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(54) Title: COMPOSITIONS AND METHODS FOR PREVENTING AND TREATING ALLOGRAFT REJECTION

(57) Abstract

Compositions and methods are provided for the prevention and treatment of allograft rejection. Compositions are provided which comprise an antisense oligonucleotide targeted to a nucleic acid sequence encoding intercellular adhesion molecule-1, vascular cell adhesion molecule-1, or endothelial leukocyte adhesion molecule-1 in combination with an immunosuppressive agent. Methods of preventing or treating allograft rejection by treating an allograft recipient with such a composition are provided. Methods for preventing allograft rejection comprising pretreatment of the graft are also provided.

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COMPOSITIONS AND METHODS FOR PREVENTING AND TREATING ALLOGRAFT REJECTION

FIELD OF THE INVENTION

This invention relates to compositions and methods for preventing and treating allograft rejection. In particular, compositions comprising an antisense oligonucleotide in combination with an immunosuppressive agent are provided. The antisense oligonucleotide is targeted to nucleic acids encoding intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1, also known as E-selectin) or vascular cell adhesion molecule-1 (VCAM-1). The immunosuppressive agent is a monoclonal antibody, antisense oligonucleotide or conventional immunosuppressive agent such as brequinar, rapamycin or antilymphocyte serum. These compositions have been found to extend allograft survival times and induce donor-specific transplant tolerance. These compositions are useful for preventing and treating allograft rejection and for inducing tolerance to specific allergens or antigens.

BACKGROUND OF THE INVENTION

Inflammation is a localized protective response elicited by tissues in response to injury, infection, or tissue destruction resulting in the destruction of the infectious or injurious agent and isolation of the injured tissue. A typical inflammatory response proceeds as follows: recognition of an antigen as foreign or recognition of tissue damage; synthesis and release of soluble inflammatory mediators; recruitment of inflammatory cells to the site of infection or tissue damage;

destruction and removal of the invading organism or damaged tissue; and deactivation of the system once the invading organism or damage has been resolved.

Cell-cell interactions are involved in the activation of the immune response at each of the stages described above. One of the earliest detectable events in a normal inflammatory response is adhesion of leukocytes to the vascular endothelium, followed by migration of leukocytes out of the vasculature to the site of infection or injury. The adhesion of these leukocytes, or white blood cells, to vascular endothelium is an obligate step in the migration out of the vasculature. Harlan, J.M., *Blood* **1985**, 65, 513-525.

The adhesion of white blood cells to vascular endothelium and other cell types is mediated by interactions between specific proteins, termed "adhesion molecules", located on the plasma membrane of both white blood cells and vascular endothelium. The interaction between adhesion molecules is similar to classical receptor ligand interactions with the exception that the ligand is fixed to the surface of a cell instead of being soluble. The adherence of white blood cells to vascular endothelium appears to be mediated in part if not *in toto* by the five cell adhesion molecules: intercellular adhesion molecule-1 (ICAM-1); ICAM-2; endothelial leukocyte adhesion molecule-1 (ELAM-1, also called E-selectin); vascular cell adhesion molecule-1 (VCAM-1); and granule membrane protein-140 (GMP-140). Expression on the cell surface of ICAM-1, ICAM-2, ELAM-1, VCAM-1 and GMP-140 adhesion molecules is induced by inflammatory stimuli. The expression of ELAM-1 and VCAM-1 on endothelial cells is induced by cytokines such as interleukin-1ß and tumor necrosis factor, but not gamma-interferon. ICAM-1 expression on endothelial cells is induced by the cytokines, interleukin-1 tumor necrosis factor and gamma-interferon.

In organ transplantation, the reaction of host immune cells with transplant cells is mediated by adhesive cell membrane receptors. An essential step in the activation of T lymphocytes is their interaction with endothelial cells of the

graft. Binding of T lymphocytes to the endothelial cells requires intercellular adhesion molecules. It is believed that the induction of ICAM-1 influences the leukocyte response in transplanted tissue. ICAM-1 has been shown to be expressed in rejecting kidney and liver allografts; Faull and Russ, *Transplantation* **1989**, 48, 226-230; Adams et al., *Lancet* **1989**, 2(8672), 1122-1125. ICAM-1 is also expressed on the endothelial-rich pancreatic islet complex; Zeng et al., *Transplantation* **1994**, 58, 681-689. Other adhesion molecules, including VCAM-1 and ELAM-1, are also known to be involved in interactions between the transplanted tissue and the immune system.

It is believed that compositions comprising inhibitors of ICAM-1, VCAM-1 and ELAM-1 expression could provide a novel therapeutic class of anti-rejection agents. The use of neutralizing monoclonal antibodies against ICAM-1 in animal models provides evidence that such inhibitors, if identified, would have therapeutic benefit for renal allografts (Cosimi et al., *J. Immunol.* **1990**, 144, 4604-4612), cardiac allografts (Isobe et al., *Science* **1992**, 255, 1125-1127) and pancreatic islet allografts and xenografts (Zeng et al., *Transplantation* **1994**, 58, 681-689). Experiments in monkeys have been performed to examine the effectiveness of monoclonal antibodies to ICAM-1 in blocking rejection of kidney allografts. Cosimi et al., *J. Immunol.* **1990**, 144, 4604-4612. As in humans, ICAM-1 molecules are expressed on vascular endothelium in normal kidney. During rejection, ICAM-1 expression increased on endothelial and tubular cells and on leukocytes; this increase correlated with massive infiltration of grafts. Treatment with monoclonal antibody to ICAM-1 decreased cellular infiltration and allowed the necessary cyclosporine A dosage to be reduced. Clinical trials conducted in high-risk kidney allograft patients showed that treatment with mouse anti-ICAM-1 monoclonal antibody in a 14-day postoperative period in addition to the triple drug therapy (cyclosporine A, azathioprine and corticosteroids) improved one-year allograft survival from 56% to 78%. Haug et al., *Transplantation* **1993**, 55, 766-773. However, the majority

of patients developed human anti-mouse antibodies within the first two weeks following completion of monoclonal treatment.

Current agents which affect intercellular adhesion molecules include synthetic peptides, monoclonal antibodies, and soluble forms of the adhesion molecules. To date, synthetic peptides which block the interactions with VCAM-1 or ELAM-1 have not been identified. Monoclonal antibodies may prove to be useful for the treatment of allograft rejection due to expression of ICAM-1, VCAM-1 and ELAM-1. The role of ICAM-1 and LFA-1 molecules in graft rejection has been previously demonstrated by treatment of heart allograft recipient mice with monoclonal antibodies to ICAM-1 and LFA-1. This combined treatment induced long-term allograft survival and donor-specific transplantation tolerance. Isobe et al., *Science* **1992**, 255, 1125-1127. However, with chronic treatment, the host animal develops an immune response against the monoclonal antibodies thereby limiting their usefulness in long-term therapy. Soluble forms of the cell adhesion molecules suffer from many of the same limitations as monoclonal antibodies in addition to the expense of their production and their low binding affinity. Thus, there is a long felt need for compositions which effectively inhibit allograft rejection.

PCT/US90/02357 (Hession et al.) discloses DNA sequences encoding Endothelial Adhesion Molecules (ELAMs), including ELAM-1 and VCAM-1 and VCAM-1b. A number of uses for these DNA sequences are provided, including (1) production of monoclonal antibody preparations that are reactive for these molecules which may be used as therapeutic agents to inhibit leukocyte binding to endothelial cells; (2) production of ELAM peptides to bind to the ELAM ligand on leukocytes which, in turn, may bind to ELAM on endothelial cells, inhibiting leukocyte binding to endothelial cells; (3) use of molecules binding to ELAMS (such as anti-ELAM antibodies, or markers such as the ligand or fragments of it) to detect inflammation; and (4) use of ELAM and ELAM ligand DNA sequences to produce nucleic acid molecules which intervene in ELAM or ELAM ligand expression at the translational level using antisense nucleic

acid and ribozymes to block translation of a specific mRNA either by masking mRNA with antisense nucleic acid or cleaving it with a ribozyme. It is disclosed that coding regions are the targets of choice. For VCAM-1, AUG is believed to be most likely; a 15-mer hybridizing to the AUG site is specifically disclosed in Example 17 of PCT/US90/02357.

SUMMARY OF THE INVENTION

In accordance with the present invention, compositions for treating allograft rejection are provided. These compositions comprise an antisense oligonucleotide which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1 in combination with an immunosuppressive agent.

Also in accordance with the present invention, methods of preventing or treating allograft rejection are provided which comprise treating an allograft recipient with an antisense oligonucleotide which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1, in combination with an immunosuppressive agent.

Further in accordance with the present invention, methods of preventing rejection of an allograft are provided which comprise treatment of the graft prior to transplantation.

DETAILED DESCRIPTION OF THE INVENTION

Recognition of an antigen as foreign is the initial step in the inflammatory response to injury, infection or tissue destruction. Allograft rejection also begins with the recognition of foreign antigens. The acute infiltration of neutrophils into the site of inflammation appears to be due to increased expression of GMP-140, ELAM-1 and ICAM-1 on the surface of endothelial cells. The appearance of lymphocytes and monocytes during the later stages of an inflammatory reaction appear to be mediated by VCAM-1 and ICAM-1. ELAM-1 and GMP-140 are transiently expressed on vascular endothelial cells, while VCAM-1 and ICAM-1 are chronically expressed.

ICAM-1 is a member of the immunoglobulin supergene family, containing 5 immunoglobulin-like domains at the amino

terminus, followed by a transmembrane domain and a cytoplasmic domain. Human ICAM-1 is encoded by a 3.3-kb mRNA resulting in the synthesis of a 55,219 dalton protein. The mRNA sequence of human ICAM-1 (SEQ ID NO: 97) was described by Staunton et al., *Cell* **1988**, 52, 925-933. The mature glycosylated protein has an apparent molecular mass of 90 kDa as determined by SDS-polyacrylamide gel electrophoresis.

ICAM-1 exhibits a broad tissue and cell distribution, and may be found on white blood cells, endothelial cells, fibroblast, keratinocytes and other epithelial cells. The expression of ICAM-1 can be regulated on vascular endothelial cells, fibroblasts, keratinocytes, astrocytes and several cell lines by treatment with bacterial lipopolysaccharide and cytokines such as interleukin-1, tumor necrosis factor, gamma-interferon, and lymphotoxin. *See, e.g.,* Frohman et al., *J. Neuroimmunol*. **1989**, 23, 117-124. Increased expression of ICAM-1 molecules correlates with increased leukocyte infiltration followed by the rejection of organ allografts in both humans and mice. Nickoloff et al., *J. Immunol*. **1993**, 150, 2148-2159.

ELAM-1 is a 115-kDa membrane glycoprotein which is a member of the selectin family of membrane glycoproteins. The mRNA sequence of human ELAM-1 (SEQ ID NO:98) was described by Bevilacqua et al., *Science* **1989**, 243, 1160-1165. The amino terminal region of ELAM-1 contains sequences with homologies to members of lectin-like proteins, followed by a domain similar to epidermal growth factor, followed by six tandem 60-amino acid repeats similar to those found in complement receptors 1 and 2. These features are also shared by GMP-140 and MEL-14 antigen, a lymphocyte homing antigen. ELAM-1 is encoded for by a 3.9-kb mRNA. The 3'-untranslated region of ELAM-1 mRNA contains several ATTTA sequence motifs which are responsible for the rapid turnover of cellular mRNA consistent with the transient nature of ELAM-1 expression.

ELAM-1 exhibits a limited cellular distribution in that it has only been identified on vascular endothelial cells. Like ICAM-1, ELAM-1 is inducible by a number of cytokines including tumor necrosis factor, interleukin-1 and lymphotoxin

and bacterial lipopolysaccharide. In contrast to ICAM-1, ELAM-1 is not induced by gamma-interferon. Bevilacqua et al., *Proc. Natl. Acad. Sci. USA* **1987**, 84, 9238-9242; Wellicome et al., *J. Immunol.* **1990**, 144, 2558-2565. The kinetics of ELAM-1 mRNA induction and disappearance in human umbilical vein endothelial cells precedes the appearance and disappearance of ELAM-1 on the cell surface.

VCAM-1 is a 110-kDa membrane glycoprotein encoded by a 3.2-kb mRNA. The sequence of human VCAM-1 mRNA (SEQ ID NO: 99) was described by Osborn et al., *Cell* **1989**, 59, 1203-1211. VCAM-1 appears to be encoded by a single-copy gene which can undergo alternative splicing to yield products with either six or seven immunoglobulin domains. The receptor for VCAM-1 is proposed to be CD29 (VLA-4) as demonstrated by the ability of monoclonal antibodies to CD29 to block adherence of Ramos cells to VCAM-1. VCAM-1 is expressed primarily on vascular endothelial cells. Like ICAM-1 and ELAM-1, expression of VCAM-1 on vascular endothelium is regulated by treatment with cytokines. Rice and Bevilacqua, *Science* **1989**, 246, 1303-1306; Rice et al., *J. Exp. Med.* **1990**, 171, 1369-1374.

The present invention employs oligonucleotides targeted to nucleic acid sequences encoding ICAM-1, VCAM-1 or ELAM-1. This relationship between an oligonucleotide and the nucleic acid sequence to which it is targeted is commonly referred to as "antisense." "Targeting" an oligonucleotide to a chosen nucleic acid target, in the context of this invention, is a multistep process. The process usually begins with identifying a nucleic acid sequence whose function is to be modulated. This may be, as examples, a cellular gene (or mRNA made from the gene) whose expression is associated with a particular disease state, or a foreign nucleic acid from an infectious agent. In the present invention, the target is a nucleic acid sequence encoding ICAM-1, VCAM-1 or ELAM-1; in other words, the gene encoding ICAM-1, VCAM-1 or ELAM-1, or mRNA expressed from the gene encoding ICAM-1, VCAM-1 or ELAM-1. The targeting process also includes determination of a site or sites within the nucleic acid sequence for the oligonucleotide

interaction to occur such that the desired effect, i.e., modulation of gene expression, will result. Once the target site or sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired modulation.

In the context of this invention "modulation" means either inhibition or stimulation. Inhibition of target gene expression is presently the preferred form of modulation. This modulation can be measured in ways which are routine in the art, for example by Northern blot assay of mRNA expression or Western blot assay of protein expression as taught in the examples of the instant application. Effects on allograft survival and graft rejection can also be measured, as taught in the examples of the instant application. "Hybridization", in the context of this invention, means hydrogen bonding, also known as Watson-Crick base pairing, between complementary bases, usually on opposite nucleic acid strands or two regions of a nucleic acid strand. Guanine and cytosine are examples of complementary bases which are known to form three hydrogen bonds between them. Adenine and thymine are examples of complementary bases which form two hydrogen bonds between them. "Specifically hybridizable" and "complementary" are terms which are used to indicate a sufficient degree of complementarity such that stable and specific binding occurs between the DNA or RNA target and the oligonucleotide. It is understood that an oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment or, in the case of *in vitro* assays, under conditions in which the assays are conducted.

In preferred embodiments of this invention, oligonucleotides are provided which are targeted to mRNA encoding ICAM-1, VCAM-1 or ELAM-1. In accordance with this invention, persons of ordinary skill in the art will understand that mRNA includes not only the coding region which carries the information to encode a protein using the three letter genetic code, but also associated ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, the 5' cap region, intron regions and intron/exon or splice junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these associated ribonucleotides as well as to the coding ribonucleotides. The functions of messenger RNA to be interfered with include all vital functions such as translocation of the RNA to the site for protein translation, actual translation of protein from the RNA, splicing or maturation of the RNA and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of such interference with the RNA function is to cause interference with ICAM-1, VCAM-1 or ELAM-1 protein expression.

In the context of this invention, the term "oligonucleotide" refers to an oligomer or polymer of nucleotide or nucleoside monomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages. The term "oligonucleotide" also includes oligomers or polymers comprising non-naturally occurring monomers, or portions thereof, which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced cellular uptake, increased stability in the presence of nucleases, or enhanced target affinity. A number of nucleotide and nucleoside modifications have been shown to make the oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide. Nuclease resistance is routinely measured by incubating oligonucleotides with cellular extracts or isolated nuclease

solutions and measuring the extent of intact oligonucleotide remaining over time, usually by gel electrophoresis. Oligonucleotides which have been modified to enhance their nuclease resistance survive intact for a longer time than unmodified oligonucleotides. A number of modifications have also been shown to increase binding (affinity) of the oligonucleotide to its target. Affinity of an oligonucleotide for its target (in this case, a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1) is routinely determined by measuring the Tm of an oligonucleotide/target pair, which is the temperature at which the oligonucleotide and target dissociate. Dissociation is detected spectrophotometrically. The higher the Tm, the greater the affinity of the oligonucleotide for the target. In some cases, oligonucleotide modifications which enhance target binding affinity are also, independently, able to enhance nuclease resistance.

Specific examples of some preferred oligonucleotides envisioned for this invention may contain phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar ("backbone") linkages. Most preferred are phosphorothioates and those with CH_2-NH-O-CH_2, CH_2-N(CH_3)-O-CH_2, CH_2-O-N(CH_3)-CH_2, CH_2-N(CH_3)-N(CH_3)-CH_2 and O-N(CH_3)-CH_2-CH_2 backbones (where phosphodiester is O-P-O-CH_2). Also preferred are oligonucleotides having morpholino backbone structures. Summerton, J.E. and Weller, D.D., U.S. Patent No: 5,034,506. In other preferred embodiments, such as the protein-nucleic acid or peptide-nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide may be replaced with a polyamide backbone, the bases being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone. P.E. Nielsen, M. Egholm, R.H. Berg, O. Buchardt, *Science* **1991**, 254, 1497. Other preferred oligonucleotides may contain alkyl and halogen-substituted sugar moieties comprising one of the following at the 2′ position: OH, SH, SCH_3, F, OCN, OCH_3OCH_3, $OCH_3O(CH_2)_nCH_3$, $O(CH_2)_nNH_2$ or $O(CH_2)_nCH_3$ where n is from 1 to about 10; C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl or

aralkyl; Cl; Br; CN; CF_3; OCF_3; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a cholesteryl group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Oligonucleotides may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group. Other preferred embodiments may include at least one modified base form or "universal base" such as inosine.

The oligonucleotides in accordance with this invention preferably are from about 8 to about 50 nucleotides in length. In the context of this invention it is understood that this encompasses non-naturally occurring oligomers as hereinbefore described, having 8 to 50 monomers.

The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the oligonucleotides is well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotides such as the phosphorothioates and alkylated derivatives. It is also well known to use similar techniques and commercially available modified amidites and controlled-pore glass (CPG) products such as those available from Glen Research, Sterling VA, to synthesize modified oligonucleotides such as cholesterol-modified oligonucleotides.

For prophylactics and therapeutics, methods of preventing and treating allograft rejection are provided. The formulation of therapeutic compositions and their subsequent administration is believed to be within the skill in the art. In accordance with some embodiments of this invention, an allograft recipient is treated by administering compositions

comprising an antisense oligonucleotide targeted to ICAM-1, VCAM-1 or ELAM-1 in combination with an immunosuppressive agent. In the context of the present invention, "in combination" means that the oligonucleotide and immunosuppressive agent are administered in the same course of treatment and may be administered separately, simultaneously or in a mixture, i.e., a single composition or formulation containing both oligonucleotide and immunosuppressive agent. Examples of immunosuppressive agents include conventional immunosuppressive agents, of which brequinar, rapamycin, and anti-lymphocyte serum are preferred, and monoclonal antibodies, of which those directed to LFA-1 or ICAM-1 are preferred. The immunosuppressive agent may also be an antisense oligonucleotide. Preferred among these are oligonucleotides targeted to B7-2 or LFA-1, or oligonucleotides targeted to ICAM-1, VCAM-1 or ELAM-1.

Oligonucleotides and/or immunosuppressive agents, or combinations of the two, may be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface active agents, liposomes or lipid formulations and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like. Formulations for parenteral administration may include sterile aqueous solutions which may also contain buffers, liposomes, diluents and other suitable additives.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration may be topical (including ophthalmic, vaginal, rectal, intranasal), oral, by inhalation, or parenteral, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. In the present invention, intraperitoneal injection, oral gavage or intravenous infusion by osmotic pump are preferred modes of administration.

Dosing is dependent on severity and responsiveness of

the condition to be treated, with course of treatment lasting from several days to several months or until a cure is effected or a diminution of disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual compositions, and can generally be estimated based on EC50's in *in vitro* and animal studies. In general, dosage is from 0.001 μg to 100 g and may be administered once or several times daily, weekly, monthly or yearly, or even every 2 to 20 years.

For prevention of allograft rejection, the allograft may be treated prior to transplantation. Perfusion of the allograft is a preferred form of treatment; *ex vivo* perfusion is more preferred. Methods of organ perfusion are well known in the art. In general, harvested tissues or organs (preferably heart, kidney or pancreas) are perfused with the compositions of the invention in a pharmacologically acceptable carrier such as, for example, lactated Ringer's solution, University of Wisconsin (UW) solution, Euro-Collins solution or Sachs solution. Simple flushing of the organ or pulsatile perfusion may be used. Perfusion time is generally dependent on the length of *ex vivo* viability of the organ being transplanted; these viability times vary from organ to organ and are known in the art. Hearts and livers, for example, are generally transplanted within 4 to 6 hours of harvesting, whereas other organs may have longer ischemic viability. Kidneys, for example, may be transplanted up to 48 hr or even 72 hr after harvesting. Dosage may range from 0.001 μg to 500 g each of oligonucleotide and immunosuppressive agent. Pancreatic islet cell allografts are now being used in place of whole pancreas transplants because of the reduced likelihood of rejection. Islet cell transplants are effective in allowing diabetic patients to become independent of insulin injections. Hering et al., *Cell Transplant* **1993**, 2, 269-282. For pancreatic islet allografts, treatment of the isolated islets

ex vivo may be preferred. Zeng et al., *Transplantation* **1994**, 58,681-689. Dosage may range from 0.001 μg to 500 g each of oligonucleotide and immunosuppressive agent.

Prophylactic treatment of the allograft recipient with oligonucleotide and/or immunosuppressive agent may also be preferred for prevention of allograft rejection. In this case dosages are expected to be from 0.0001 μg to 100 g each of oligonucleotide and immunosuppressive agent.

Several preferred embodiments of this invention are exemplified in accordance with the following nonlimiting examples. Persons of ordinary skill in the art will appreciate that the present invention is not so limited, however, and that it is generally applicable.

EXAMPLES

Example 1 Synthesis and characterization of oligonucleotides

Unmodified DNA oligonucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine. ß-cyanoethyldiisopropyl-phosphoramidites were purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle was replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step was increased to 68 seconds and was followed by the capping step.

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides were purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel electrophoresis was accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, pH 7.0. Oligodeoxynucleotides and phosphorothioate oligonucleotides were judged from electrophoresis to be greater than 80% full length material.

The relative amounts of phosphorothioate and

phosphodiester linkages obtained by this synthesis were periodically checked by ^{31}P NMR spectroscopy. The spectra were obtained at ambient temperature using deuterium oxide or dimethyl sulfoxide-d_6 as solvent. Phosphorothioate samples typically contained less than one percent of phosphodiester linkages.

Secondary evaluation was performed with oligonucleotides purified by trityl-on HPLC on a PRP-1 column (Hamilton Co., Reno, Nevada) using a gradient of acetonitrile in 50 mM triethylammonium acetate, pH 7.0 (4% to 32% in 30 minutes, flow rate = 1.5 ml/min). Appropriate fractions were pooled, evaporated and treated with 5% acetic acid at ambient temperature for 15 minutes. The solution was extracted with an equal volume of ethyl acetate, neutralized with ammonium hydroxide, frozen and lyophilized. HPLC-purified oligonucleotides were not significantly different in potency from precipitated oligonucleotides, as judged by the ELISA assay for ICAM-1 expression.

Example 2 Quantitation of ICAM-1, VCAM-1 and ELAM-1 expression by ELISA

Expression of ICAM-1, VCAM-1 and ELAM-1 on the surface of cells was quantitated using specific monoclonal antibodies in an ELISA. Cells were grown to confluence in 96-well microtiter plates. The cells were stimulated with either interleukin-1 or tumor necrosis factor for 4 to 8 hours to quantitate ELAM-1 and 8 to 24 hours to quantitate ICAM-1 and VCAM-1. Following the appropriate incubation time with the cytokine, the cells were gently washed three times with a buffered isotonic solution containing calcium and magnesium such as Dulbecco's phosphate buffered saline (D-PBS). The cells were then directly fixed on the microtiter plate with 1 to 2% paraformaldehyde diluted in D-PBS for 20 minutes at 25°C. The cells were washed again with D-PBS three times. Nonspecific binding sites on the microtiter plate were blocked with 2% bovine serum albumin in D-PBS for 1 hour at 37°C. Cells were incubated with the appropriate monoclonal antibody diluted in blocking solution for 1 hour at 37°C. Unbound

antibody was removed by washing the cells three times with D-PBS. Antibody bound to the cells was detected by incubation with a 1:1000 dilution of biotinylated goat anti-mouse IgG (Bethesda Research Laboratories, Gaithersberg, MD) in blocking solution for 1 hour at 37°C. Cells were washed three times with D-PBS and then incubated with a 1:1000 dilution of streptavidin conjugated to ß-galactosidase (Bethesda Research Laboratories) for 1 hour at 37°C. The cells were washed three times with D-PBS for 5 minutes each. The amount of ß-galactosidase bound to the specific monoclonal antibody was determined by developing the plate in a solution of 3.3 mM chlorophenolred-ß-D-galactopyranoside, 50 mM sodium phosphate, 1.5 mM $MgCl_2$; pH=7.2 for 2 to 15 minutes at 37°C. The concentration of the product was determined by measuring the absorbance at 575 nm in an ELISA microtiter plate reader.

Induction of ICAM-1 was observed following stimulation with either interleukin-1ß or tumor necrosis factor α in several human cell lines. Cells were stimulated with increasing concentrations of interleukin-1 or tumor necrosis factor for 15 hours and processed as described above. ICAM-1 expression was determined by incubation with a 1:1000 dilution of the monoclonal antibody 84H10 (Amac Inc., Westbrook, ME). The cell lines used were passage 4 human umbilical vein endothelial cells (HUVEC), a human epidermal carcinoma cell line (A431), a human melanoma cell line (SK-MEL-2) and a human lung carcinoma cell line (A549). ICAM-1 was induced on all the cell lines; however, tumor necrosis factor was more effective than interleukin-1 in induction of ICAM-1 expression on the cell surface.

Screening antisense oligonucleotides for inhibition of ICAM-1, VCAM-1 or ELAM-1 expression was performed as described above with the exception of pretreatment of cells with the oligonucleotides prior to challenge with the cytokines. Human umbilical vein endothelial cells (HUVEC) were treated with increasing concentration of oligonucleotide diluted in Opti MEM (GIBCO, Grand Island, NY) containing 8 μM N-[1-(2,3-dioleyloxy) propyl]-N,N,N-trimethylammonium chloride

(DOTMA) for 4 hours at 37°C to enhance uptake of the oligonucleotides. The medium was removed and replaced with endothelial growth medium (EGM-UV; Clonetics, San Diego, CA) containing the indicated concentration of oligonucleotide for an additional 4 hours. Interleukin-1β was added to the cells at a concentration of 5 units/ml and incubated for 14 hours at 37°C. The cells were quantitated for ICAM-1 expression using a 1:1000 dilution of the monoclonal antibody 84H10 as described above. The oligonucleotides used were:

COMPOUND 1 - (ISIS 1558) a phosphodiester oligonucleotide targeted to position 64-80 of the mRNA covering the AUG initiation of translation codon having the sequence

5'-TGGGAGCCATAGCGAGGC-3' (SEQ ID NO: 1).

COMPOUND 2 - (ISIS 1570) a phosphorothioate oligonucleotide corresponding to the same sequence as COMPOUND 1.

COMPOUND 3 - a phosphorothioate oligonucleotide complementary to COMPOUND 1 and COMPOUND 2 exhibiting the sequence

5'-GCCTCGCTATGGCTCCCA-3' (SEQ ID NO: 81).

COMPOUND 4 - (ISIS 1572) a phosphorothioate oligonucleotide targeted to positions 2190-2210 of the mRNA in the 3' untranslated region containing the sequence

5'-GACACTCAATAAATAGCTGGT-3' (SEQ ID NO: 3).

COMPOUND 5 - (ISIS 1821) a phosphorothioate oligonucleotide targeted to human 5-lipoxygenase mRNA used as a control containing the sequence

5'-CATGGCGCGGGCCGCGGG-3' (SEQ ID NO: 82).

The phosphodiester oligonucleotide targeting the AUG initiation of translation region of the human ICAM-1 mRNA (COMPOUND 1) did not inhibit expression of ICAM-1; however, the corresponding phosphorothioate oligonucleotide (COMPOUND 2) inhibited ICAM-1 expression by 70% at a concentration of 0.1 μM and 90% at 1 μM concentration. The increased potency of the phosphorothioate oligonucleotide over the phosphodiester is due to increased stability. The sense strand to COMPOUND 2, COMPOUND 3, inhibited ICAM-1 expression by 25% at 10 μM. If

COMPOUND 2 was prehybridized to COMPOUND 3 prior to addition to the cells, the effects of COMPOUND 2 on ICAM-1 expression were attenuated suggesting that the activity of COMPOUND 2 was due to antisense oligonucleotide effect, requiring hybridization to the mRNA. The antisense oligonucleotide directed against 3′ untranslated sequences (COMPOUND 4) inhibited ICAM-1 expression by 62% at a concentration of 1 μM. The control oligonucleotide, targeting human 5-lipoxygenase (COMPOUND 5), reduced ICAM-1 expression by 20%. These data demonstrate that oligonucleotides are capable of inhibiting ICAM-1 expression on human umbilical vein endothelial cells and suggest that the inhibition of ICAM-1 expression is due to an antisense activity.

The antisense oligonucleotide COMPOUND 2 at a concentration of 1 μM was shown to inhibit expression of ICAM-1 on human umbilical vein endothelial cells stimulated with either tumor necrosis factor or interleukin-1. These data demonstrate that the effects of COMPOUND 2 are not specific for stimulation of cells by a particular cytokine.

Example 3 Cell adherence assay

A second cellular assay which was used to demonstrate the effects of antisense oligonucleotides on ICAM-1, VCAM-1 or ELAM-1 expression was a cell adherence assay. Target cells were grown as a monolayer in a multiwell plate, treated with oligonucleotide followed by cytokine. The adhering cells were then added to the monolayer cells and incubated for 30 to 60 minutes at 37°C and washed to remove nonadhering cells. Cells adhering to the monolayer may be determined either by directly counting the adhering cells or prelabeling the cells with a radioisotope such as ^{51}Cr and quantitating the radioactivity associated with the monolayer as described. Dustin and Springer, *J. Cell Biol*. **1988**, 107, 321-331.

An example of the effects of antisense oligonucleotides targeting ICAM-1 mRNA on the adherence of DMSO differentiated HL-60 cells to tumor necrosis factor treated human umbilical vein endothelial cells is as follows. Human

umbilical vein endothelial cells were grown to 80% confluence in 12 well plates. The cells were treated with 2 μM oligonucleotide diluted in Opti-MEM containing 8 μM DOTMA for 4 hours at 37°C. The medium was removed and replaced with fresh endothelial cell growth medium (EGM-UV) containing 2 μM of the indicated oligonucleotide and incubated 4 hours at 37°C. Tumor necrosis factor, 1 ng/ml, was added to cells as indicated and cells incubated for an additional 19 hours. The cells were washed once with EGM-UV and 1.6 x 10^6 HL-60 cells differentiated for 4 days with 1.3% DMSO added. The cells were allowed to attach for 1 hour at 37°C and gently washed 4 times with Dulbecco's phosphate-buffered saline (D-PBS) warmed to 37°C. Adherent cells were detached from the monolayer by addition of 0.25 ml of cold (4°C) phosphate-buffered saline containing 5 mM EDTA and incubated on ice for 5 minutes. The number of cells removed by treatment with EDTA was determined by counting with a hemocytometer. Endothelial cells detached from the monolayer by EDTA treatment could easily be distinguished from HL-60 cells by morphological differences.

In the absence of tumor necrosis factor, 3% of the HL-60 cells bound to the endothelial cells. Treatment of the endothelial cell monolayer with 1 ng/ml tumor necrosis factor increased the number of adhering cells to 59% of total cells added. Treatment with the antisense oligonucleotide COMPOUND 2 or the control oligonucleotide COMPOUND 5 did not change the number of cells adhering to the monolayer in the absence of tumor necrosis factor treatment. The antisense oligonucleotide, COMPOUND 2, reduced the number of adhering cells from 59% of total cells added to 17% of the total cells added. In contrast, the control oligonucleotide, COMPOUND 5, did not significantly reduce the number of cells adhering to the tumor necrosis factor treated endothelial monolayer, i.e., 53% of total cells added for COMPOUND 5 treated cells versus 59% for control cells.

These data indicate that antisense oligonucleotides are capable of inhibiting ICAM-1 expression on endothelial cells and that inhibition of ICAM-1 expression correlates with

a decrease in the adherence of a neutrophil-like cell to the endothelial monolayer in a sequence specific fashion. Because other molecules, such as ELAM-1 and VCAM-1, also mediate adherence of white blood cells to endothelial cells, it is not expected that adherence would be completely blocked by antisense to ICAM-1.

Example 4 Cell culture and treatment with oligonucleotides

The human lung carcinoma cell line A549 was obtained from the American Type Culture Collection (Bethesda MD). Cells were grown in Dulbecco's Modified Eagle's Medium (Irvine Scientific, Irvine CA) containing 1 gm glucose/liter and 10% fetal calf serum (Irvine Scientific). Human umbilical vein endothelial cells (HUVEC) (Clonetics, San Diego CA) were cultured in EGM-UV medium (Clonetics). HUVEC were used between the second and sixth passages. Human epidermal carcinoma A431 cells were obtained from the American Type Culture Collection and cultured in DMEM with 4.5 g/l glucose. Primary human keratinocytes were obtained from Clonetics and grown in KGM (Keratinocyte growth medium, Clonetics).

Cells grown in 96-well plates were washed three times with Opti-MEM (GIBCO, Grand Island, NY) prewarmed to 37°C. 100 μl of Opti-MEM containing either 10 μg/ml N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA, Bethesda Research Labs, Bethesda MD) in the case of HUVEC cells or 20 μg/ml DOTMA in the case of A549 cells was added to each well. Oligonucleotides were sterilized by centrifugation through 0.2 μm Centrex cellulose acetate filters (Schleicher and Schuell, Keene, NH). Oligonucleotides were added as 20x stock solution to the wells and incubated for 4 hours at 37°C. Medium was removed and replaced with 150 μl of the appropriate growth medium containing the indicated concentration of oligonucleotide. Cells were incubated for an additional 3 to 4 hours at 37°C then stimulated with the appropriate cytokine for 14 to 16 hours, as indicated. ICAM-1 expression was determined as described in Example 2. The presence of DOTMA during the first 4 hours incubation with oligonucleotide

increased the potency of the oligonucleotides at least 100-fold. This increase in potency correlated with an increase in cell uptake of the oligonucleotide.

Example 5 ELISA screening of additional antisense oligonucleotides for activity against ICAM-1 gene expression in Interleukin-1ß-stimulated cells

Antisense oligonucleotides were originally targeted to five sites on the human ICAM-1 mRNA. Oligonucleotides were synthesized in both phosphodiester (P=O; ISIS 1558, 1559, 1563, 1564 and 1565) and phosphorothioate (P=S; ISIS 1570, 1571, 1572, 1573, and 1574) forms. The oligonucleotides are shown in Table 1.

TABLE 1

ANTISENSE OLIGONUCLEOTIDES WHICH TARGET HUMAN ICAM-1

ISIS NO.	SEQ ID NO.	TARGET REGION	MODIFICATION
1558	1	AUG Codon (64-81)	P=O
1559	2	5'-Untranslated (32-49)	P=O
1563	3	3'-Untranslated (2190-3010)	P=O
1564	4	3'-Untranslated (2849-2866)	P=O
1565	5	Coding Region (1378-1395)	P=O
1570	1	AUG Codon (64-81)	P=S
1571	2	5'-Untranslated (32-49)	P=S
1572	3	3'-Untranslated (2190-3010)	P=S
1573	4	3'-Untranslated (2849-2866)	P=S
1574	5	Coding Region (1378-1395)	P=S
1930	6	5'-Untranslated (1-20)	P=S
1931	7	AUG Codon (55-74)	P=S
1932	8	AUG Codon (72-91)	P=S
1933	9	Coding Region (111-130)	P=S
1934	10	Coding Region (351-370)	P=S
1935	11	Coding Region (889-908)	P=S

1936	12	Coding Region (1459-1468)	P=S
1937	13	Termination Codon (1651-1687)	P=S
1938	14	Termination Codon (1668-1687)	P=S
1939	15	3'-Untranslated (1952-1971)	P=S
1940	16	3'-Untranslated (2975-2994)	P=S
2149	17	AUG Codon (64-77)	P=S
2163	18	AUG Codon (64-75)	P=S
2164	19	AUG Codon (64-73)	P=S
2165	20	AUG Codon (66-80)	P=S
2173	21	AUG Codon (64-79)	P=S
2302	22	3'-Untranslated (2114-2133)	P=S
2303	23	3'-Untranslated (2039-2058)	P=S
2304	24	3'-Untranslated (1895-1914)	P=S
2305	25	3'-Untranslated (1935-1954)	P=S
2307	26	3'-Untranslated (1976-1995)	P=S
2634	1	AUG-Codon (64-81)	2'-fluoro A, C & U; P=S
2637	15	3'-Untranslated (1952-1971)	2'-fluoro A, C & U;
2691	1	AUG Codon (64-81)	P=O, except last 3 bases, P=S
2710	15	3'-Untranslated (1952-1971)	2'-O-methyl; P=O
2711	1	AUG Codon (64-81)	2'-O-methyl; P=O
2973	15	3'-Untranslated (1952-1971)	2'-O-methyl; P=S
2974	1	AUG Codon (64-81)	2'-O-methyl; P=S

3064	27	5'-CAP (32-51)	P=S; G & C added as spacer to 3'
3067	84	5'-CAP (32-51)	P=S
3222	84	5'-CAP (32-51)	2'-O-methyl; P=O
3224	84	5'-CAP (32-51)	2'-O-methyl; P=S
3581	85	3'-Untranslated (1959-1978)	P=S

Based on the initial data obtained with the five original targets, additional oligonucleotides targeted to the ICAM-1 mRNA were tested. The antisense oligonucleotide (ISIS 3067) which is targeted to the predicted transcription initiation site (5' cap site) inhibited ICAM-1 expression by nearly 90% in IL-1ß-stimulated cells. ISIS 1931 and 1932 are targeted 5' and 3', respectively, to the AUG translation initiation codon. All three oligonucleotides targeted to the AUG region inhibit ICAM-1 expression, though ISIS 1932 yielded approximately 20% inhibition and thus was less active than ISIS 1570 (70% inhibition) or ISIS 1931 (>50% inhibition). Oligonucleotides targeted to the coding region of ICAM-1 mRNA (ISIS 1933, 1934, 1935, 1574 and 1936) exhibited weak activity. Oligonucleotides targeted to the translation termination codon (ISIS 1937 and 1938) exhibited moderate activity, e.g., over 50% inhibition in the case of ISIS 1938.

Surprisingly, the most active antisense oligonucleotide was ISIS 1939, a phosphorothioate oligonucleotide targeted to a sequence in the 3'- untranslated region of ICAM-1 mRNA (see Table 1). This oligonucleotide gave complete inhibition of ICAM-1 expression. Oligonucleotides targeted to other 3' untranslated sequences (ISIS 1572, 1573 and 1940) were not as active as ISIS 1939.

Because ISIS 1939 unexpectedly exhibited the greatest antisense activity of the original 16 oligonucleotides tested, other oligonucleotides targeted to sequences in the 3'-untranslated region of ICAM-1 mRNA (ISIS 2302, 2303, 2304,

2305, and 2307, as shown in Table 1) were tested. ISIS 2307, which is targeted to a site only five bases 3' to the ISIS 1939 target, was the least active of the series, and still showed nearly 70% inhibition of ICAM expression. ISIS 2302, which is targeted to the ICAM-1 mRNA at a position 143 bases 3' to the ISIS 1939 target, was the most active of the series, with nearly 100% inhibition. Examination of the predicted RNA secondary structure of the human ICAM-1 mRNA 3'-untranslated region (according to M. Zuker, *Science* **1989**, 244, 48-52) revealed that both ISIS 1939 and ISIS 2302 are targeted to sequences predicted to be in a stable stem-loop structure. However, it is generally believed that regions of RNA secondary structure should be avoided when designing antisense oligonucleotides. Thus, ISIS 1939 and ISIS 2302 would not have been predicted to inhibit ICAM-1 expression.

The control oligonucleotide ISIS 1821 showed a small amount of activity against ICAM expression, probably due in part to its ability to hybridize (12 of 13 base match) to the ICAM-1 mRNA at a position 15 bases 3' to the AUG translation initiation codon.

These studies indicate that the AUG translation initiation codon and specific 3'-untranslated sequences in the ICAM-1 mRNA were the most susceptible to antisense oligonucleotide inhibition of ICAM-1 expression.

In addition to inhibiting ICAM-1 expression in human umbilical vein cells and the human lung carcinoma cells (A549), ISIS 1570, ISIS 1939 and ISIS 2302 were shown to inhibit ICAM-1 expression in primary human keratinocytes by nearly 70%, over 80% and over 80%, respectively. These oligonucleotides also inhibited ICAM-1 expression in the human epidermal carcinoma A431 cells. These data demonstrate that antisense oligonucleotides are capable of inhibiting ICAM-1 expression in several human cell lines. Furthermore, the rank order potency of the oligonucleotides is the same in the four cell lines examined.

Example 6 Specificity of antisense inhibition of ICAM-1

The specificity of the antisense oligonucleotides ISIS 1570 and ISIS 1939 for ICAM-1 was evaluated by immunoprecipitation of ^{35}S-labelled proteins. A549 cells were grown to confluence in 25 cm^2 tissue culture flasks and treated with antisense oligonucleotides as described in Example 4. The cells were stimulated with interleukin-1ß for 14 hours, washed with methionine-free DMEM plus 10% dialyzed fetal calf serum, and incubated for 1 hour in methionine-free medium containing 10% dialyzed fetal calf serum, 1 μM oligonucleotide and interleukin-1ß as indicated. ^{35}S-Methionine/cysteine mixture (Tran^{35}S-label, purchased from ICN, Costa Mesa, CA) was added to the cells to an activity of 100 μCi/ml and the cells were incubated an additional 2 hours. Cellular proteins were extracted by incubation with 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1.0% NP-40, 0.5% deoxycholate and 2 mM EDTA (0.5 ml per well) at 4°C for 30 minutes. The extracts were clarified by centrifugation at 18,000 x g for 20 minutes. The supernatants were preadsorbed with 200 μl protein G-Sepharose beads (Bethesda Research Labs, Bethesda MD) for 2 hours at 4°C, divided equally and incubated with either 5 μg ICAM-1 monoclonal antibody (purchased from AMAC Inc., Westbrook ME) or HLA-A,B antibody (W6/32, produced by murine hybridoma cells obtained from the American Type Culture Collection, Bethesda, MD) for 15 hours at 4°C. Immune complexes were trapped by incubation with 200 μl of a 50% suspension of protein G-Sepharose (v/v) for 2 hours at 4°C, washed 5 times with lysis buffer and resolved on an SDS-polyacrylamide gel. Proteins were detected by autoradiography.

Treatment of A549 cells with 5 units/ml of interleukin-1ß was shown to result in the synthesis of a 95-100 kDa protein migrating as a doublet which was immunoprecipitated with the monoclonal antibody to ICAM-1. The appearance as a doublet is believed to be due to differently glycosylated forms of ICAM-1. Pretreatment of the cells with the antisense oligonucleotide ISIS 1570 at a concentration of 1 μM decreased the synthesis of ICAM-1 by approximately 50%, while 1 μM ISIS 1939 decreased

ICAM-1 synthesis to near background. Antisense oligonucleotide ISIS 1940, inactive in the ICAM-1 ELISA assay (Examples 2 and 5) did not significantly reduce ICAM-1 synthesis. None of the antisense oligonucleotides targeted to the ICAM-1 gene had a demonstrable effect on HLA-A, B synthesis, demonstrating the specificity of the oligonucleotides for ICAM-1. Furthermore, the proteins which nonspecifically precipitated with the ICAM-1 antibody and protein G-Sepharose were not significantly affected by treatment with the antisense oligonucleotides.

Example 7 Screening of additional antisense oligonucleotid s for activity against ICAM-1 by cell adhesion assay

Human umbilical vein endothelial (HUVEC) cells were grown and treated with oligonucleotides as in Example 4. Cells were treated with either ISIS 1939, ISIS 1940, or the control oligonucleotide ISIS 1821 for 4 hours, then stimulated with TNF-α for 20 hours. Basal HUVEC minimally bound HL-60 cells, while TNF-stimulated HUVEC bound 19% of the total cells added. Pretreatment of the HUVEC monolayer with 0.3 μM ISIS 1939 reduced the adherence of HL-60 cells to basal levels. The control oligonucleotide, ISIS 1821, and ISIS 1940 reduced the percentage of cells adhering from 19% to 9%. These data indicate that antisense oligonucleotides targeting ICAM-1 can specifically decrease adherence of a leukocyte-like cell line (HL-60) to TNF-α-treated HUVEC.

Example 8 ELISA screening of antisense oligonucleotides for activity against ELAM-1 gene expression

Primary human umbilical vein endothelial (HUVEC) cells, passage 2 to 5, were plated in 96-well plates and allowed to reach confluence. Cells were washed three times with Opti-MEM (GIBCO, Grand Island NY). Cells were treated with increasing concentrations of oligonucleotide diluted in Opti-MEM containing 10 μg/ml DOTMA solution (Bethesda Research Labs, Bethesda MD) for 4 hours at 37°C. The medium was removed and replaced with EGM-UV (Clonetics, San Diego CA) plus oligonucleotide. Tumor necrosis factor α was added to the medium (2.5 ng/ml) and the cells were incubated an additional

4 hours at 37°C.

ELAM-1 expression was determined by ELISA. Cells were gently washed three times with Dulbecco's phosphate-buffered saline (D-PBS) prewarmed to 37°C. Cells were fixed with 95% ethanol at 4°C for 20 minutes, washed three times with D-PBS and blocked with 2% BSA in D-PBS. Cells were incubated with ELAM-1 monoclonal antibody BBA-1 (R&D Systems, Minneapolis MN) diluted to 0.5 μg/ml in D-PBS containing 2% BSA for 1 hour at 37°C. Cells were washed three times with D-PBS and the bound ELAM-1 antibody detected with biotinylated goat anti-mouse secondary antibody followed by ß-galactosidase-conjugated streptavidin as described in Example 2.

The activity of antisense phosphorothioate oligonucleotides which target 11 different regions on the ELAM-1 cDNA and two oligonucleotides which target ICAM-1 (as controls) was determined using the ELAM-1 ELISA. The oligonucleotide and targets are shown in Table 2.

TABLE 2

ANTISENSE OLIGONUCLEOTIDES WHICH TARGET HUMAN ELAM-1

ISIS NO.	SEQ ID NO.	TARGET REGION	MODIFICATION
1926	28	AUG Codon (143-164)	P=S
2670	29	3'-Untranslated (3718-3737)	P=S
2673	30	3'-Untranslated (2657-2677)	P=S
2674	31	3'-Untranslated (2617-2637)	P=S
2678	32	3'-Untranslated (3558-3577)	P=S
2679	33	5'-Untranslated (41-60)	P=S
2680	34	3'-Untranslated (3715-3729)	P=S
2683	35	AUG Codon (143-163)	P=S
2686	36	AUG Codon (149-169)	P=S
2687	37	5'-Untranslated (18-37)	P=S
2693	38	3'-Untranslated (2760-2788)	P=S
2694	39	3'-Untranslated (2934-2954)	P=S

In contrast to what was observed with antisense oligonucleotides targeted to ICAM-1 (Example 5), the most potent oligonucleotide modulator of ELAM-1 activity (ISIS 2679)

was targeted to a specific sequence in the 5'-untranslated region of ELAM-1. This oligonucleotide completely inhibited ELAM-1 expression. ISIS 2687, an oligonucleotide which targeted to sequences ending three bases upstream of the ISIS 2679 target, showed only 10-15% inhibition. Therefore, ISIS 2679 is targeted to a site on the ELAM-1 mRNA, which is sensitive to inhibition with antisense oligonucleotides. The sensitivity of this site to inhibition with antisense oligonucleotides was not predictable based upon RNA secondary structure predictions or information in the literature.

Example 9 ELISA screening of additional antisens oligonucleotides for activity against ELAM-1 gene expression

Inhibition of ELAM-1 expression by eighteen antisense phosphorothioate oligonucleotides was determined using the ELISA assay as described in Example 8. The sequence and activity of each oligonucleotide against ELAM-1 are shown in Table 3. The oligonucleotides indicated by an asterisk (*) have IC50's of approximately 50 nM or below and are preferred. IC50 indicates the dosage of oligonucleotide which results in 50% inhibition of ELAM-1 expression. An additional oligonucleotide targeted to the 3'-untranslated region (ISIS 4728) did not inhibit ELAM expression.

TABLE 3

Inhibition of human ELAM-1 expression by antisense oligonucleotides
ELAM-1 expression is given as % of control

ISIS#	SEQ ID#	POSITION		SEQUENCE	VCAM-1 EXPRESSION 30 nM oligo	50 nM oligo
*4764	52	5'-UTR	1-19	GAAGTCAGCCAAGAACAGCT	70.2	50.2
2687	37	5'-UTR	17-36	TATAGGAGTTTTGATGTGAA	91.1	73.8
*2679	33	5'-UTR	40-59	CTGCTGCCTCTGTCTCAGGT	6.4	6.0
*4759	53	5'-UTR	64-83	ACAGGATCTCTCAGGTGGGT	30.0	20.2
*2683	35	AUG	143-163	AATCATGACTTCAAGAGTTCT	47.9	48.5
*2686	36	AUG	148-168	TGAAGCAATCATGACTTCAAG	51.1	46.9
*4756	54	I/E	177-196	CCAAAGTGAGAGCTGAGAGA	53.9	35.7
4732	55	Coding	1936-1955	CTGATTCAAGGCTTTGGCAG	68.5	55.3
*4730	56	I/E 3'UTR	2006-2025	TTCCCCAGATGCACCTGTTT	14.1	2.3
*4729	57	3'-UTR	2063-2082	GGGCCAGAGACCCGAGGAGA	49.4	46.3
*2674	31	3'-UTR	2617-2637	CACAATCCTTAAGAACTCTTT	33.5	28.1
2673	30	3'-UTR	2656-2676	GTATGGAAGATTATAATATAT	58.9	53.8
2694	39	3'-UTR	2933-2953	GACAATATACAAACCTTCCAT	72.0	64.6
*4719	58	3'-UTR	2993-3012	ACGTTTGGCCTCATGGAAGT	36.8	34.7
4720	59	3'-UTR	3093-3112	GGAATGCAAAGCACATCCAT	63.5	70.6
*2678	32	3'-UTR	3557-3576	ACCTCTGCTGTTCTGATCCT	24.9	15.3
2670	29	3'-UTR	3717-3736	ACCACACTGGTATTTCACAC	72.2	67.2

I/E indicates Intron/Exon junction

Oligonucleotides with IC_{50}'s of approximately 50 nM or below are indicated by an asterisk (*).

Exampl 10 ELISA screening of antis nse oligonucleotid s for activity against VCAM-1 gene expression

Inhibition of VCAM-1 expression by fifteen antisense phosphorothioate oligonucleotides was determined using the ELISA assay approximately as described in Example 8, except that cells were stimulated with TNF-α for 16 hours and VCAM-1 expression was detected by a VCAM-1 specific monoclonal antibody (R & D Systems, Minneapolis, MN) used at 0.5 μg/ml. The sequence and activity of each oligonucleotide against VCAM-1 are shown in Table 4. The oligonucleotides indicated by an asterisk (*) have IC_{50}'s of approximately 50 nM or below and are preferred. IC_{50} indicates the dosage of oligonucleotide which results in 50% inhibition of VCAM-1 expression.

TABLE 4

Inhibition of human VCAM-1 expression by antisense oligonucleotides
VCAM-1 expression is given as % of control

ISIS#	SEQ ID#	POSITION		SEQUENCE	VCAM-1 EXPRESSION 30 nM oligo	50 nM oligo
*5884	60	5'-UTR	1-19	CGATGCAGATACCGCGGAGT	79.2	37.2
3791	61	5'-UTR	38-58	GCCTGGGAGGGTATTCAGCT	92.6	58.0
5862	62	5'-UTR	48-68	CCTGTGTGTGCCTGGGAGGG	115.0	83.5
*3792	63	AUG	110-129	GGCATTTTAAGTTGCTGTCG	68.7	33.7
5863	64	CODING	745-764	CAGCCTGCCTTACTGTGGGC	95.8	66.7
*5874	65	CODING	1032-1052	CTTGAACAATTAATTCCACCT	66.5	35.3
5885	66	E/I	1633-1649+intron	TTACCATTGACATAAAGTGTT	84.4	52.4
*5876	67	CODING	2038-2057	CTGTGTCTCCTGTCTCCGCT	43.5	25.6
*5875	68	CODING	2183-2203	GTCTTTGTTGTTTTCTCTTCC	59.2	34.8
3794	69	TERMIN.	2344-2362	TGAACATATCAAGCATTAGC	75.3	52.6
*3800	70	3'-UTR	2620-2639	GCAATCTTGCTATGGCATAA	64.4	47.7
*3805	71	3'-UTR	2826-2845	CCCGGCATCTTTACAAAACC	67.7	44.9
*3801	50	3'-UTR	2872-2892	AACCCAGTGCTCCCTTTGCT	36.5	21.3
*5847	72	3'-UTR	2957-2976	AACATCTCCGTACCATGCCA	51.8	24.6
*3804	51	3'-UTR	3005-3024	GGCCACATTGGGAAAGTTGC	55.1	23.3

E/I indicates exon/intron junction

Oligonucleotides with IC_{50}'s of approximately 50 nM or below are indicated by an asterisk (*).

Exampl 11 Murine models for testing antis nse oligonucleotides against ICAM-1

Many conditions which are believed to be mediated by intercellular adhesion molecules are not amenable to study in humans. For example, allograft rejection is a condition which is likely to be ameliorated by interference with ICAM-1 expression, but clearly this must be evaluated in animals rather than human transplant patients. These conditions can be tested in animal models, however, such as the mouse models used here.

Oligonucleotide sequences for inhibiting ICAM-1 expression in murine cells were identified. Murine ICAM-1 has approximately 50% homology with the human ICAM-1 sequence; a series of oligonucleotides which target the mouse ICAM-1 mRNA sequence were designed and synthesized, using information gained from evaluation of oligonucleotides targeted to human ICAM-1. These oligonucleotides were screened for activity using an immunoprecipitation assay.

Murine DCEK-ICAM-1 cells (a gift from Dr. Adrienne Brian, University of California at San Diego) were treated with 1 μM of oligonucleotide in the presence of 20 μg/ml DOTMA/DOPE solution for 4 hours at 37°C. The medium was replaced with methionine-free medium plus 10% dialyzed fetal calf serum and 1 μM antisense oligonucleotide. The cells were incubated for 1 hour in methionine-free medium, then 100 μCi/ml ^{35}S-labeled methionine/cysteine mixture was added to the cells. Cells were incubated an additional 2 hours, washed 4 times with PBS, and extracted with buffer containing 20 mM Tris, pH 7.2, 20 mM KCl, 5 mM EDTA, 1% Triton X-100, 0.1 mM leupeptin, 10 μg/ml aprotinin, and 1 mM PMSF. ICAM-1 was immunoprecipitated from the extracts by incubating with a murine-specific ICAM-1 antibody (YN1/1.7.4) followed by protein G-sepharose. The immunoprecipitates were analyzed by SDS-PAGE and autoradiographed. Phosphorothioate oligonucleotides ISIS 3066 and 3069, which target the AUG codon of mouse ICAM-1, inhibited ICAM-1 synthesis by 48% and 63%, respectively, while oligonucleotides ISIS 3065 and ISIS 3082, which target sequences in the 3'-untranslated region of murine ICAM-1 mRNA

inhibited ICAM-1 synthesis by 47% and 97%, respectively. The most active antisense oligonucleotide against mouse ICAM-1 was targeted to the 3'-untranslated region. ISIS 3082 was evaluated further based on these results; this 20-mer phosphorothioate oligonucleotide comprises the sequence (5' to 3') TGC ATC CCC CAG GCC ACC AT (SEQ ID NO: 83).

Example 12 Evaluation of ICAM-1 antisense oligonucleotides in bEND.3 murine endothelioma cells

bEND.3 cells were provided by Dr. Werner Risau, Max-Planck-Instiutes, Martinsreid, Germany. Cells were treated with oligonucleotide in the presence of 15 μg/ml DOTMA/DOPE liposome formulation for 4 hours. ICAM-1 expression was induced by treatment with 5 ng/ml human rTNF-α and 1000 u/ml murine IFN-γ for 16 hours. Cells were fixed with ethanol and ICAM-1 expression was quantitated by incubating with ICAM-1 monoclonal antibody (YN1/1.7.4, purified from ascites) followed by a biotinylated goat anti-rat IgG antibody and streptavidin-conjugated ß-galactosidase. Results are expressed as percent control ICAM-1 expression. Both basal and cytokine-treated cells were pretreated with DOTMA.

Phosphorothioate oligonucleotides ISIS 3068, 3069, 3066, 3070, 3065, 3082, 3806, 3083, 3084 and 3099 were screened by ELISA in the bEND.3 murine endothelioma cell line. These oligonucleotides are shown in Table 5.

TABLE 5

Effect of antisense phosphorothioate oligonucleotides on ICAM-1 expression in bEND.3 cells

ISIS#	Sequence	% of control expression	SEQ ID NO
3068	AGC TGC GCT GCT ACC TGC AC	25%	89
3069	GCC CAT TGC AGG GCC AGG GC	-5%	87
3066	GGG TTG AAG CCA TTG CAG GG	45%	86
3070	CTC ATC CAG CAG GCT CAG GG	75%	90
3065	CCA GAG GAA GTG GCT GAG GG	35%	88
3082	TGC ATC CCC CAG GCC ACC AT	-55%	83
3806	CAA GTG TGC ATC CCC CAG GC	-30%	91
3083	TTG GGA CAA TGT CTC AGC TT	25%	92
3084	TGC CAG TCC ACA TAG TGT TT	25%	93
3099	TGC TTA CCC TCC CAC AGC AG	5%	94

The bEND.3 cells expressed a basal level of ICAM-1 molecules that increased significantly after treatment with a combination of human TNF-α and murine IFN-γ. All of the oligonucleotides inhibited cytokine-induced ICAM-1 expression compared to control, two oligonucleotides, ISIS 3082 and ISIS 3806, lowered ICAM-1 protein expression to below the basal level of expression. ISIS 3082 was also shown to reduce cytokine-induced ICAM-1 mRNA by greater than 95%. This effect was specific. Control oligonucleotide ISIS 7253 (SEQ ID NO: 95, a random mixture of the four bases at each position in a phosphorothioate 20 mer) and unrelated control oligonucleotide ISIS 1082 (SEQ ID NO: 96) did not reduce ICAM-1 mRNA expression.

Example 13 Antisense oligonucleotide to ICAM-1 increases survival in murine heterotopic heart transplant model

To determine the therapeutic effects of ICAM-1 antisense oligonucleotide in preventing allograft rejection, the murine ICAM-1 specific oligonucleotide ISIS 3082 was tested for activity in a murine vascularized heterotopic heart transplant

model. Hearts from Balb/c mice were transplanted into the abdominal cavity of C3H mice as primary vascularized grafts essentially as described by Isobe et al., *Circulation* **1991**, 84, 1246-1255. Oligonucleotides were administered by continuous intravenous administration via a 7-day Alzet pump. The mean survival time for untreated mice was 9.2 ± 0.8 days (8, 9, 9, 9, 10, 10 days). Treatment of the mice for 7 days with 5 mg/kg ISIS 3082 increased the mean survival time to 14.3 ± 4.6 days (11, 12, 13, 21 days).

Example 14 Additional mouse heterotopic heart transplants:

Other donor/recipient combinations were found to give similar results in the cardiac allograft experiments. Untreated C3H(H-2)^k mice rejected C57BL/10(H-2)^b vascularized heart allografts at a mean survival time of 7.7 ± 1.4 days (6, 7, 7, 7, 8, 9, 10 days). A 7-day infusion of the unrelated control oligonucleotide, ISIS 1082, at either 5.0 or 10.0 mg/kg/day did not affect allograft survival (7.1 ± 0.7 days). In contrast, infusion of the ICAM-1 antisense oligonucleotide ISIS 3082 prolonged allograft survival in a dose-dependent fashion: 1.25 mg/kg/day prolonged graft survival to 11.0 ± 0 days; 2.5 mg/kg/day prolonged survival to 12.0 ± 2.7 days (9, 10, 12, 13, 16 days), 5.0 mg/kg/day to 14.1 ± 2.7 days (10, 12, 12, 13, 16, 16, 17, 17 days); and 10.0 mg/kg/day to 15.3 ± 5.8 days (12, 12, 13, 24 days). All are $p < 0.01$. Extended 14-day treatment with ISIS 3082 (5 mg/kg/day) further increased graft survival up to as much as 30 days (16, 17, 29, 30; mean = 23.0 ± 7.5 days). Similar results were obtained with C57BL/6(H-2^b) to BALB/c transplants.

The effectiveness of the immunosuppression was documented by histological examination of the grafts on day 6 after transplantation. Syngeneic C57BL/10 hearts transplanted to C57BL/10 recipients showed mild infiltration with mononuclear cells (10% of the myocardium) compared to normal controls. Heart allografts from untreated recipients displayed strong infiltration with mononuclear cells and neutrophils. This effect was associated with severe necrosis and mineralization that

formed a dense band that affected 60% of the epicardium, myocardium and papillary muscles. In contrast, heart allografts from recipients treated with ISIS 3082 (5 mg/kg/day) showed only scattered infiltration with mononuclear cells in 20% of the myocardium. The antisense oligonucleotide targeted to ICAM-1 inhibited infiltration and subsequent destruction of heart allograft tissue by host cells.

Example 15 Antisense oligonucleotide to ICAM-1 combin d with monoclonal antibody to LFA-1 increases survival indefinitely in murine heterotopic heart transplant model

Monoclonal antibody (MAb-LFA-1) to LFA-1 was obtained from Dr. Yogita, Juntendo University School of Medicine, Tokyo, Japan. C3H recipients of C57 BL/10 hearts were untreated or treated with daily i.p. injection for 7 days of MAb-LFA-1 (50 μg/day) alone or in combination with ISIS 3082 (5.0 mg/kg/day, administered by Alzet osmotic pump for 7 days). Treatment with MAb-LFA-1 alone prolonged allograft survival to 14.3 ± 2.7 days. Combined treatment with MAb-LFA-1 and ISIS 3082 for 7 days resulted in indefinite survival of the heart allografts (>150 days; $p < 0.001$) in all 5 mice so treated. The interaction between two agents (oligonucleotide and immunosuppressant) was assessed by the combination index (CI) method (Chou, T-C. and Talalay, P. *Adv. Enz. Regul.* **1984**, 22, 27) for the doses to achieve x% inhibition (days of graft survival):

$$CIx = \frac{D_1 \text{ combined}}{(Dx)_1 \text{ alone}} + \frac{D_2 \text{ combined}}{(Dx)_2 \text{ alone}}$$

for the mutually exclusive case where both drugs have the same or similar modes of action, or the more conservative expression:

$$CIx = \frac{D_1 \text{ combined}}{(Dx)_1 \text{ alone}} + \frac{D_1 \text{ combined}}{(Dx)_2 \text{ alone}} + \frac{(D_1 \text{ combined})\ (D_2 \text{ combined})}{[(Dx)_1 \text{ alone}]\ [(Dx_2 \text{ alone}]}$$

for the mutually exclusive case, where each drug has a different mode of action. Computer software (Biosoft, Cambridge UK) was used to determine the CI values. A CI of 1 indicates an additive effect, CI < 1 indicates a synergistic effect and CI > 1

indicates an antagonistic effect.

The CI value calculated for the combination of 5.0 mg/kg/day ISIS 3082 and 50 μg/day anti-LFA-1 monoclonal antibody was 0.001, indicating strong synergism.

Example 16 Antisense oligonucleotide to ICAM-1 combined with monoclonal antibody to LFA-1 induces donor-specific transplantation tolerance

Recipients bearing C57BL/10 hearts for 65 days (n=4) were transplanted with donor-type C57BL/10 and third-party BALB/c (H-2^d) skin allografts. Induction of transplantation tolerance was demonstrated by permanent acceptance of donor-type skin grafts (>100 days) and acute rejection of third-party grafts in 9.0 ± 0.0 days. Control C3H mice (n=5) rejected C57BL/10 and BALB/c grafts in 9.2 ± 0.8 days and 8.1 ± 0.6 days, respectively. These results indicate that the combination of ICAM-1 antisense oligonucleotide and monoclonal antibody to LFA-1 induces donor-specific transplantation tolerance.

Example 17 Effects of antisense oligonucleotide to ICAM-1 combined with conventional immunosuppressive drugs

The interaction of ISIS 3082 with the immunosuppressive agents rapamycin (RAPA), brequinar (BQR), cyclosporine A (CsA) and anti-lymphocyte serum (ALS) was examined. CsA (Sandoz, Basel, Switzerland) dissolved in cremophor (Sigma, St. Louis MO) was delivered via jugular venous infusion by a 7-day osmotic pump (Alzet, Palo Alto CA). RAPA (Wyeth Ayerst, Rouse Point NY) diluted in 10% Tween 80, 20% N-N-dimethylacetamide and 70% PEG-400 was infused i.v. by 7-day osmotic pump. BQR (DuPont, Wilmington DE) diluted in distilled water was administered every second day, q.o.d, by oral gavage for 7 days. Rabbit anti-mouse ALS (Accurate, New York, NY) was injected once i.p. two days before grafting.

These immunosuppressive modalities act in different ways: ALS decreases the level of T cells, including the alloantigen-specific T cells. Monaco et al., *J. Immunol.* **1966**, 96, 229-238. RAPA inhibits the transduction of signals delivered by lymphokines (Morice et al., *J. Biol. Chem.* **1993**, 268, 3734-

3738) and BQR blocks the dehydroorotate dehydrogenase enzyme that is required for pyrimidine synthesis [Chen et al., *Cancer Res.* **1986**, 46, 5014-5020]. CsA blocks calcineurin activity, thereby inhibiting the synthesis of lymphokines by T cells. Liu et al., *Cell* **1991**, 66, 807-815.

A single i.p. injection of ALS alone two days prior to transplantation prolonged graft survival in a dose-dependent manner: 0.1 ml gave a mean survival of 9.0 ± 0.0 days; 0.2 ml gave a mean of 10.4 ± 0.5 days (10, 10, 10, 11, 11 days) and 0.4 ml gave a mean survival of 14.0 ± 2.1 days (11, 14, 15, 16 days). All are $p < 0.01$. The combination of 0.2 ml ALS and the antisense oligonucleotide ISIS 3082 extended allograft survivals to 32.2 ± 8.3 days (20, 30, 31, 39, 41 days), 37.0 ± 5.8 days (32, 32, 41, 43 days) and 72.0 ± 49.1 days (33, 34, 54, 89, >150 days), respectively. All are $p < 0.01$ and CI < 0.001.

RAPA alone (0.05, 0.1 or 0.2 mg/kg/day) delivered i.v. by a 7-day osmotic pump prolonged graft survival in a dose-dependent manner: 0.05 mg/kg/day gave a mean survival of 7.4 ± 1.4 days (6, 6, 7, 9, 9 days); 0.1 mg/kg/day gave a mean survival of 13.0 ± 7.5 days (10, 11, 20, 20, 21 days) and 0.2 mg/kg/day gave a mean survival of 20.0 ± 10.9 days (12, 14, 17, 18, 39 days). The combination of 0.1 mg/kg/day RAPA and the antisense oligonucleotide ISIS 3082 extended allograft survivals to 32.4 ± 8.9 days (23, 24, 33, 39, 43 days) at 5 mg/kg/day of ISIS 3082 and 36.3 ± 6.1 days (32, 32, 36, 45 days) at 10 mg/kg/day of ISIS 3082. Both are $p < 0.01$ and CI < 0.02.

Oral gavage with BQR alone (0.5, 1.0 or 2.0 mg/kg/day) delivered every second day (q.o.d.) for 7 days prolonged allograft survival to 12.0 ± 2.4 days (9, 11, 11, 14, 15 days), 17.6 days (13, 16, 18, 19, 22 days) or 20.0 ± 4.1 days (15, 17, 20, 23, 25 days), respectively. The combination of 0.5 mg/kg BQR and 5.0 mg/kg ISIS 3082 resulted in a mean survival time of 38.8 ± 30.2 days (21, 24, 28, 28, 31, >100) ($p < 0.01$; CI = 0.007).

A 7-day i.v. infusion of CsA, 2.5 or 5.0 mg/kg/day, was ineffective; 10.0 or 20.0 mg/kg/day CsA did prolong allograft survival. Addition of ISIS 3082 (5.0 or 10.0 mg/kg/day) to CsA

treatment (5.0 mg/kg/day) did not improve graft survival. CI was 14.1 and 51.0, respectively. A combination of the control oligonucleotide, ISIS 1082, and CsA did not affect graft survival time.

These results show that the ICAM-1 antisense oligonucleotide ISIS 3082 interacts synergistically with the immunosuppressive agents ALS, RAPA and BQR, but not with CsA, to block allograft rejection. Because CsA is not very effective in mice, it is unclear whether the lack of synergism between the antisense oligonucleotide and CsA is a pharmacological or a pharmacokinetic effect.

Example 18 *Toxicology and pharmacokinetics of ISIS 3082*

The ICAM-1 antisense oligonucleotide ISIS 3082 was well tolerated at therapeutic doses without producing signs of toxicity. Even at high doses (100.0 mg/kg/day given q.o.d for 14 days), ISIS 3082 did not produce any major side effects and did not induce an antigenic response.

Interestingly, ISIS 3082 was shown to be active in prolonging heart allograft survival when delivered in a saline suspension, without cationic liposomes. Similar observations have been made with other phosphorothioate oligonucleotides directed at other targets (see, for example, Simons et al., *Nature* **1992**, 359, 67-70; Kitajima et al., *Science* **1992**, 258, 1792-1795). Thus, although cationic liposomes enhance the effect of many oligonucleotides, including ISIS 3082, *in vitro*, they are not necessarily required for efficacy of the same oligonucleotides *in vivo*.

Example 19 Mouse pancreatic islet transplants

Fully H-2 and non-H-2 incompatible C3H ($H-2^K$) streptozotocin-induced diabetic mice were transplanted with 700 fresh C57 BL/10 ($H-2^b$) dextran gradient-purified islet cells, into either the renal subcapsular space or embolized through the portal vein to the liver. All animals analyzed had non-fasting blood sugars less than 200 mg/dl within 4 post-operative days. The day of rejection was defined as the first day of two

consecutive blood sugars >300 mg/dl and was documented histologically.

Glucose tolerance tests were done at postoperative days 2 and 7. After a 4-hour fast, the control and oligonucleotide-treated groups were given 2 grams dextrose/kg body weight IP. Blood sugars were recorded at 0, 15, 30, 45 and 90 minutes.

Example 20 Effect of anti-ICAM-1 oligonucleotide ISIS 3082 or monoclonal antibodies on pancreatic islet graft survival and islet function

Graft survival: There were four treatment groups following kidney capsule transplantation. 1) Mice receiving no immunosuppressive treatment (control) had a mean survival time (MST) ± standard deviation of 10.7 ± 2.3 days. 2) Mice treated with anti-LFA-1 monoclonal antibody, 50 mg daily IP for 7 days had a MST of 27.2 ± 4.8 days, $p < 0.01$. 3) Mice treated with anti- ICAM-1 monoclonal antibody YN1/1.7.4, 100 μg IP daily for 7 days, had a MST of 21.9 ± 2.0 days, $p < 0.01$. 4) Mice treated with anti-ICAM-1 oligonucleotide ISIS 3082 (SEQ ID NO: 83), 5 mg/kg/day IP via osmotic pump had a MST of 28.9 ± 12 days, $p < 0.01$.

After portal vein administration, control mice survived 11.2 ± 2.6 days and ISIS 3082 oligonucleotide-treated mice had a MST of 30.0 ± 18 days, $p < 0.01$.

Glucose tolerance tests: On postoperative day 2, the oligonucleotide-treated group had lower mean blood sugars compared to controls at 30 minutes (142.6 ± 72 vs. 231.3 ± 53.8, $p < 0.05$) and 45 minutes (100.4 ± 68.4 vs. 199.5 ± 62.1, $p < 0.5$). On postoperative day 7, the oligonucleotide-treated group also had lower mean blood sugars compared to controls at 30 minutes (189 ± 58.5 vs. 251.5 ± 70.1, $p < 0.05$) and 45 minutes 148.6 ± 40.2 vs. 210.7 ± 58.2, $p < 0.5$).

Significant islet allograft prolongation was achieved by ICAM-1 blockade. ICAM-1 antisense oligonucleotide was effective in improving islet function as well as prolonging graft survival.

Example 21 Identification of rat oligonucleotides *in vitro*

Oligonucleotide sequences for inhibiting rat ICAM-1 expression were identified and screened in rat L2 cells. The most active sequence, ISIS 9125 (SEQ ID NO: 100), displayed an EC_{50} of approximately 150 nm. Sense and scrambled control sequences had no activity at doses from 150 nm to 1 μM.

Example 22 Rat kidney allografts

Kidneys from Lewis rats were transplanted into ACI rats. Control rats (no oligonucleotide treatment) had a mean graft survival time of 8.5 $\pm$ 1.0 days (7, 8, 8, 9, 9, 10 days). Rats treated with oligonucleotide ISIS 9125 alone (10 mg/kg per day) for 7 days had a mean graft survival time of 9.2 $\pm$ 1.3 days (8, 9, 9, 11 days). Rats treated with oligonucleotide ISIS 9125 alone (10 mg/kg per day) for 14 days had a mean graft survival time of >18.3 days (18, >7, >30 days).

Example 23 Rat kidney allografts with cyclosporin

Kidneys from Lewis rats were transplanted into ACI rats. Control rats (no oligo, no cyclosporin) had a mean graft survival time of 8.5 $\pm$ 1.0 days (7, 8, 8, 9, 9, 9, 10 days). Cyclosporin alone (2 mg/kg daily for 7 days) increased graft survival time to 10.5 $\pm$ 3.4 days (7, 9, 11, 15 days). Rats treated with oligonucleotide ISIS 9125 alone (10 mg/kg per day for 7 days) had a mean graft survival time of 9.25 days (8, 9, 9, 11 days). Rats treated with both cyclosporin (2 mg/kg x 7 days) and oligonucleotide ISIS 9125 (10 mg/kg x 7 days) had a mean graft survival time of >24.2 days (10, 12, 24, 30, >45 days). Treatment with a reduced cyclosporin dose of 1 mg/kg for 14 days (no oligonucleotide) gave a mean graft survival time of >17.0 days (15, 18, >18). This cyclosporin regimen in combination with ISIS 9125 (10 mg/kg, 14 days) gave a mean graft survival time of >30 days (>30, >30, >30).

Exampl 24 Rat cardiac allografts

Hearts from Lewis rats were transplanted into ACI rats using a modification of the method described in Example 12.

Control rats (no oligonucleotide treatment) had a mean graft survival time of 8.8 ± 0.8 days (8, 8, 9, 9, 9, 10 days). Rats treated with oligonucleotide ISIS 9125 alone (2.5 mg/kg for 7 days) had a mean graft survival time of 12.0 ± 1.7 days (10, 13, 13 days), rats treated with oligonucleotide ISIS 9125 alone (5 mg/kg for 7 days) had a mean graft survival time of 10 ± 3.0 days (7, 10, 13 days) and rats treated with ISIS 9125 alone (10 mg/kg per day for 7 days) had a mean graft survival time of 18.0 ± 3.8 days (13, 16, 16, 18, 22, 23 days).

Example 25 Rat cardiac allografts with cyclosporin

Hearts from Lewis rats were transplanted into ACI rats as above. Control rats (no oligo, no cyclosporin) had a mean graft survival time of 8.8 ± 0.8 days (8, 8, 9, 9, 9, 10 days). Cyclosporin alone (2 mg/kg daily for 7 days) increased graft survival time to 13.7 ± 1.5 days (12, 14, 15 days) and cyclosporine alone (4 mg/kg for 7 days) gave a graft survival time of 16.7 ± 3.8 days (14, 15, 21 days). Rats treated with oligonucleotide ISIS 9125 alone (5 mg/kg for 7 days) had a mean graft survival time of 10 ± 3.0 days (7, 10, 13 days) and rats treated with ISIS 9125 alone (10 mg/kg per day for 7 days) had a mean graft survival time of 18.0 ± 3.8 days (13, 16, 16, 18, 22, 23 days). Rats treated with both cyclosporin (4 mg/kg x 7 days) and oligonucleotide ISIS 9125 (10 mg/kg x 7 days) had a mean graft survival time of 21.7 ± 7.4 days (16, 19, 30 days).

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TGGGAGCCAT AGCGAGGC 18

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GAGGAGCTCA GCGTCGACTG 20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GACACTCAAT AAATAGCTGG T 21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GAGGCTGAGG TGGGAGGA 18

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

CGATGGGCAG TGGGAAAG 18

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GGGCGCGTGA TCCTTATAGC 20

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

CATAGCGAGG CTGAGGTTGC 20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

CGGGGGCTGC TGGGAGCCAT 20

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AGAGCCCCGA GCAGGACCAG 20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGCCCATCAG GGCAGTTTGA 20

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GGTCACACTG ACTGAGGCCT 20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CTCGCGGGTG ACCTCCCCTT 20

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

TCAGGGAGGC GTGGCTTGTG 20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

CCTGTCCCGG GATAGGTTC A 20

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CCCCCACCAC TTCCCCTCTC 20

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TTGAGAAAGC TTTATTAACT 20

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AGCCATAGCG AGGC 14

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CCATAGCGAG GC 12

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

ATAGCGAGGC 10

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TGGGAGCCAT AGCGAG 16

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGAGCCATAG CGAGGC 16

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GCCCAAGCTG GCATCCGTCA 20

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TCTGTAAGTC TGTGGGCCTC 20

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

AGTCTTGCTC CTTCCTCTTG 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CTCATCAGGC TAGACTTTAA 20

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

TGTCCTCATG GTGGGGCTAT 20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

TCTGAGTAGC AGAGGAGCTC GA 22

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CAATCATGAC TTCAAGAGTT CT 22

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ACCACACTGG TATTTCACAC 20

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GTATGGAAGA TTATAATATA T 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CACAATCCTT AAGAACTCTT T 21

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

ACCTCTGCTG TTCTGATCCT 20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CTGCTGCCTC TGTCTCAGGT 20

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

GGTATTTGAC ACAGC 15

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

AATCATGACT TCAAGAGTTC T 21

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

TGAAGCAATC ATGACTTCAA G 21

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

TATAGGAGTT TTGATGTGAA 20

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

ACAATGAGGG GGTAATCTAC A 21

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

GACAATATAC AAACCTTCCA T 21

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CCAGGCATTT TAAGTTGCTG T 21

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

CCTGAAGCCA GTGAGGCCCG 20

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

GATGAGAAAA TAGTGGAACC A 21

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

CTGAGCAAGA TATCTAGAT 19

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

CTACACTTTT GATTTCTGT 19

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

TTGAACATAT CAAGCATTAG CT 22

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

TTTACATATG TACAAATTAT GT 22

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

AATTATCACT TTACTATACA AA 22

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

AGGGCTGACC AAGACGGTTG T 21

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

CCATCTTCCC AGGCATTTTA 20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

AACCCAGTGC TCCCTTTGCT 20

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GGCCACATTG GGAAAGTTGC 20

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

GAAGTCAGCC AAGAACAGCT 20

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ACAGGATCTC TCAGGTGGGT 20

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

CCAAAGTGAG AGCTGAGAGA 20

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

CTGATTCAAG GCTTTGGCAG 20

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

TTCCCCAGAT GCACCTGTTT 20

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

GGGCCAGAGA CCCGAGGAGA 20

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

ACGTTTGGCC TCATGGAAGT 20

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

GGAATGCAAA GCACATCCAT 20

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

CGATGCAGAT ACCGCGGAGT 20

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

GCCTGGGAGG GTATTCAGCT 20

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

CCTGTGTGTG CCTGGGAGGG 20

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

GGCATTTTAA GTTGCTGTCG 20

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

CAGCCTGCCT TACTGTGGGC 20

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

CTTGAACAAT TAATTCCACC T 21

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

TTACCATTGA CATAAAGTGT T 21

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

CTGTGTCTCC TGTCTCCGCT 20

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

GTCTTTGTTG TTTTCTCTTC C 21

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

TGAACATATC AAGCATTAGC 20

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GCAATCTTGC TATGGCATAA 20

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

CCCGGCATCT TTACAAAACC 20

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

AACATCTCCG TACCATGCCA 20

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

TCACTGCTGC CTCTGTCTCA GG 22

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

TGATTCTTTT GAACTTAAAA GGA 23

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

TTAAAGGATG TAAGAAGGCT 20

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

CATAAGCACA TTTATTGTC 19

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

TTTTGGGAAG CAGTTGTTCA 20

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

AACTGTGAAG CAATCATGAC T 21

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

CCTTGAGTGG TGCATTCAAC CT 22

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

AATGCTTGCT CACACAGGCA TT 22

(2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

GCCTCGCTAT GGCTCCCA 18

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

CATGGCGCGG GCCGCGGG 18

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

TGCATCCCCC AGGCCACCAT 20

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

TCTGAGTAGC AGAGGAGCTC 20

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

TATGTCTCCC CCACCACTTC 20

(2) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

GGGTTGAAGC CATTGCAGGG 20

(2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

GCCCATTGCA GGGCCAGGGC 20

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

CCAGAGGAAG TGGCTGAGGG 20

(2) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

AGCTGCGCTG CTACCTGCAC 20

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

CTCATCCAGC AGGCTCAGGG 20

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

CAAGTGTGCA TCCCCCAGGC 20

(2) INFORMATION FOR SEQ ID NO: 92:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

TTGGGACAAT GTCTCAGCTT 20

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

TGCCAGTCCA CATAGTGTTT 20

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

TGCTTACCCT CCCACAGCAG 20

(2) INFORMATION FOR SEQ ID NO: 95:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

NNNNNNNNNN NNNNNNNNNN 20

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

GCCGAGGTCC ATGTCGTACG C 21

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3016

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

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GCTATAAGGA TCACGCGCCC CAGTCGACGC TGAGCTCCTC TGCTACTCAG      50
AGTTGCAACC TCAGCCTCGC T ATG GCT CCC                         80
                        MET ALA PRO
AGC AGC CCC CGG CCC GCG CTG CCC GCA CTC CTG GTC CTG CTC GGG 125
SER SER PRO ARG PRO ALA LEU PRO ALA LEU LEU VAL LEU LEU GLY
    5                   10                  15
GCT CTG TTC CCA GGA CCT GGC AAT GCC CAG ACA TCT GTG TCC CCC 170
ALA LEU PHE PRO GLY PRO GLY ASN ALA GLN THR SER VAL SER PRO
    20                  25                  30
TCA AAA GTC ATC CTG CCC CGG GGA GGC TCC GTG CTG GTG ACA TGC 215
SER LYS VAL ILE LEU PRO ARG GLY GLY SER VAL LEU VAL THR CYS
    35                  40                  45
AGC ACC TCC TGT GAC CAG CCC AAG TTG TTG GGC ATA GAG ACC CCG 260
SER THR SER CYS ASP GLN PRO LYS LEU LEU GLY ILE GLU THR PRO
    50                  55                  60
TTG CCT AAA AAG GAG TTG CTC CTG CCT GGG AAC AAC CGG AAG GTG 305
LEU PRO LYS LYS GLU LEU LEU LEU PRO GLY ASN ASN ARG LYS VAL
    65                  70                  75
TAT GAA CTG AGC AAT GTG CAA GAA GAT AGC CAA CCA ATG TGC TAT 350
TYR GLU LEU SER ASN VAL GLN GLU ASP SER GLN PRO MET CYS TYR
    80                  85                  90
TCA AAC TGC CCT GAT GGG CAG TCA ACA GCT AAA ACC TTC CTC ACC 395
SER ASN CYS PRO ASP GLY GLN SER THR ALA LYS THR PHE LEU THR
    95                  100                 105

GTG TAC TGG ACT CCA GAA CGG GTG GAA CTG GCA CCC CTC CCC TCT 440
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VAL TYR TRP THR PRO GLU ARG VAL GLU LEU ALA PRO LEU PRO SER
    110                 115                 120
TGG CAG CCA GTG GGC AAG AAC CTT ACC CTA CGC TGC CAG GTG GAG 485
TRP GLN PRO VAL GLY LYS ASN LEU THR LEU ARG CYS GLN VAL GLU
    125                 130                 135
GGT GGG GCA CCC CGG GCC AAC CTC ACC GTG GTG CTG CTC CGT GGG 530
GLY GLY ALA PRO ARG ALA ASN LEU THR VAL VAL LEU LEU ARG GLY
    140                 145                 150
GAG AAG GAG CTG AAA CGG GAG CCA GCT GTG GGG GAG CCC GCT GAG 575
GLU LYS GLU LEU LYS ARG GLU PRO ALA VAL GLY GLU PRO ALA GLU
    155                 160                 165
GTC ACG ACC ACG GTG CTG GTG AGG AGA GAT CAC CAT GGA GCC AAT 620
VAL THR THR THR VAL LEU VAL ARG ARG ASP HIS HIS GLY ALA ASN
    170                 175                 180
TTC TCG TGC CGC ACT GAA CTG GAC CTG CGG CCC CAA GGG CTG GAG 665
PHE SER CYS ARG THR GLU LEU ASP LEU ARG PRO GLN GLY LEU GLU
    185                 190                 195
CTG TTT GAG AAC ACC TCG GCC CCC TAC CAG CTC CAG ACC TTT GTC 710
LEU PHE GLU ASN THR SER ALA PRO TYR GLN LEU GLN THR PHE VAL
    200                 205                 210
CTG CCA GCG ACT CCC CCA CAA CTT GTC AGC CCC CGG GTC CTA GAG 755
LEU PRO ALA THR PRO PRO GLN LEU VAL SER PRO ARG VAL LEU GLU
    215                 220                 225
GTG GAC ACG CAG GGG ACC GTG GTC TGT TCC CTG GAC GGG CTG TTC 800
VAL ASP THR GLN GLY THR VAL VAL CYS SER LEU ASP GLY LEU PHE
    230                 235                 240
CCA GTC TCG GAG GCC CAG GTC CAC CTG GCA CTG GGG GAC CAG AGG 845
PRO VAL SER GLU ALA GLN VAL HIS LEU ALA LEU GLY ASP GLN ARG
    245                 250                 255
TTG AAC CCC ACA GTC ACC TAT GGC AAC GAC TCC TTC TCG GCC AAG 890
LEU ASN PRO THR VAL THR TYR GLY ASN ASP SER PHE SER ALA LYS
    260                 265                 270
GCC TCA GTC AGT GTG ACC GCA GAG GAC GAG GGC ACC CAG CGG CTG 935
ALA SER VAL SER VAL THR ALA GLU ASP GLU GLY THR GLN ARG LEU
    275                 280                 285
ACG TGT GCA GTA ATA CTG GGG AAC CAG AGC CAG GAG ACA CTG CAG 980
THR CYS ALA VAL ILE LEU GLY ASN GLN SER GLN GLU THR LEU GLN
    290                 295                 300
ACA GTG ACC ATC TAC AGC TTT CCG GCG CCC AAC GTG ATT CTG ACG 1025
THR VAL THR ILE TYR SER PHE PRO ALA PRO ASN VAL ILE LEU THR
    305                 310                 315
AAG CCA GAG GTC TCA GAA GGG ACC GAG GTG ACA GTG AAG TGT GAG 1070
LYS PRO GLU VAL SER GLU GLY THR GLU VAL THR VAL LYS CYS GLU
    320                 325                 330
GCC CAC CCT AGA GCC AAG GTG ACG CTG AAT GGG GTT CCA GCC CAG 1115
ALA HIS PRO ARG ALA LYS VAL THR LEU ASN GLY VAL PRO ALA GLN
    335                 340                 345
CCA CTG GGC CCG AGG GCC CAG CTC CTG CTG AAG GCC ACC CCA GAG 1160
PRO LEU GLY PRO ARG ALA GLN LEU LEU LEU LYS ALA THR PRO GLU
    350                 355                 360
GAC AAC GGG CGC AGC TTC TCC TGC TCT GCA ACC CTG GAG GTG GCC 1205
ASP ASN GLY ARG SER PHE SER CYS SER ALA THR LEU GLU VAL ALA
    365                 370                 375
GGC CAG CTT ATA CAC AAG AAC CAG ACC CGG GAG CTT CGT GTC CTG 1250
GLY GLN LEU ILE HIS LYS ASN GLN THR ARG GLU LEU ARG VAL LEU
    380                 385                 390
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TAT GGC CCC CGA CTG GAC GAG AGG GAT TGT CCG GGA AAC TGG ACG 1295
TYR GLY PRO ARG LEU ASP GLU ARG ASP CYS PRO GLY ASN TRP THR
    395                 400                 405
TGG CCA GAA AAT TCC CAG CAG ACT CCA ATG TGC CAG GCT TGG GGG 1340
TRP PRO GLU ASN SER GLN GLN THR PRO MET CYS GLN ALA TRP GLY
    410                 415                 420
AAC CCA TTG CCC GAG CTC AAG TGT CTA AAG GAT GGC ACT TTC CCA 1385
ASN PRO LEU PRO GLU LEU LYS CYS LEU LYS ASP GLY THR PHE PRO
    425                 430                 435
CTG CCC ATC GGG GAA TCA GTG ACT GTC ACT CGA GAT CTT GAG GGC 1430
LEU PRO ILE GLY GLU SER VAL THR VAL THR ARG ASP LEU GLU GLY
    440                 445                 450
ACC TAC CTC TGT CGG GCC AGG AGC ACT CAA GGG GAG GTC ACC CGC 1475
THR TYR LEU CYS ARG ALA ARG SER THR GLN GLY GLU VAL THR ARG
    455                 460                 465
GAG GTG ACC GTG AAT GTG CTC TCC CCC CGG TAT GAG ATT GTC ATC 1520
GLU VAL THR VAL ASN VAL LEU SER PRO ARG TYR GLU ILE VAL ILE
    470                 475                 480
ATC ACT GTG GTA GCA GCC GCA GTC ATA ATG GGC ACT GCA GGC CTC 1565
ILE THR VAL VAL ALA ALA ALA VAL ILE MET GLY THR ALA GLY LEU
    485                 490                 495
AGC ACG TAC CTC TAT AAC CGC CAG CGG AAG ATC AAG AAA TAC AGA 1610
SER THR TYR LEU TYR ASN ARG GLN ARG LYS ILE LYS LYS TYR ARG
    500                 505                 510
CTA CAA CAG GCC CAA AAA GGG ACC CCC ATG AAA CCG AAC ACA CAA 1655
LEU GLN GLN ALA GLN LYS GLY THR PRO MET LYS PRO ASN THR GLN
    515                 520                 525
GCC ACG CCT CCC TGA                                         1670
ALA THR PRO PRO ***
    530

ACCTATCCCG GGACAGGGCC TCTTCCTCGG CCTTCCCATA TTGGTGGCAG     1720

TGGTGCCACA CTGAACAGAG TGGAAGACAT ATGCCATGCA GCTACACCTA     1770

CCGGCCCTGG GACGCCGGAG GACAGGGCAT TGTCCTCAGT CAGATACAAC     1820

AGCATTTGGG GCCATGGTAC CTGCACACCT AAAACACTAG GCCACGCATC     1870

TGATCTGTAG TCACATGACT AAGCCAAGAG GAAGGAGCAA GACTCAAGAC     1920

ATGATTGATG GATGTTAAAG TCTAGCCTGA TGAGAGGGGA AGTGGTGGGG     1970

GAGACATAGC CCCACCATGA GGACATACAA CTGGGAAATA CTGAAACTTG     2020

CTGCCTATTG GGTATGCTGA GGCCCACAGA CTTACAGAAG AAGTGGCCCT     2070

CCATAGACAT GTGTAGCATC AAAACACAAA GGCCCACACT TCCTGACGGA     2120

TGCCAGCTTG GGCACTGCTG TCTACTGACC CCAACCCTTG ATGATATGTA     2170

TTTATTCATT TGTTATTTTA CCAGCTATTT ATTGAGTGTC TTTTATGTAG     2220

GCTAAATGAA CATAGGTCTC TGGCCTCACG GAGCTCCCAG TCCATGTCAC     2270

ATTCAAGGTC ACCAGGTACA GTTGTACAGG TTGTACACTG CAGGAGAGTG     2320
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CCTGGCAAAA AGATCAAATG GGGCTGGGAC TTCTCATTGG CCAACCTGCC 2370

TTTCCCCAGA AGGAGTGATT TTTCTATCGG CACAAAAGCA CTATATGGAC 2420

TGGTAATGGT TCACAGGTTC AGAGATTACC CAGTGAGGCC TTATTCCTCC 2470

CTTCCCCCCA AAACTGACAC CTTTGTTAGC CACCTCCCCA CCCACATACA 2520

TTTCTGCCAG TGTTACAATG ACACTCAGCG GTCATGTCTG GACATGAGTG 2570

CCCAGGGAAT ATGCCCAAGC TATGCCTTGT CCTCTTGTCC TGTTTGCATT 2620

TCACTGGGAG CTTGCACTAT TGCAGCTCCA GTTTCCTGCA GTGATCAGGG 2670

TCCTGCAAGC AGTGGGGAAG GGGGCCAAGG TATTGGAGGA CTCCCTCCCA 2720

GCTTTGGAAG GGTCATCCGC GTGTGTGTGT GTGTGTATGT GTAGACAAGC 2770

TCTCGCTCTG TCACCCAGGC TGGAGTGCAG TGGTGCAATC ATGGTTCACT 2820

GCAGTCTTGA CCTTTTGGGC TCAAGTGATC CTCCCACCTC AGCCTCCTGA 2870

GTAGCTGGGA CCATAGGCTC ACAACACCAC ACCTGGCAAA TTTGATTTTT 2920

TTTTTTTTTT TCAGAGACGG GGTCTCGCAA CATTGCCCAG ACTTCCTTTG 2970

TGTTAGTTAA TAAAGCTTTC TCAACTGCCA AAAAAAAAAA AAAAAA 3016

(2) INFORMATION FOR SEQ ID NO: 98:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3858

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

TTCACATCAA AACTCCTATA CTGACCTGAG ACAGAGGCAG CAGTGATACC 50

CACCTGAGAG ATCCTGTGTT TGAACAACTG CTTCCCAAAA CGGAAAGTAT 100

TTCAAGCCTA AACCTTTGGG TGAAAAGAAC TCTTGAAGTC ATG ATT 146
MET ILE

GCT TCA CAG TTT CTC TCA GCT CTC ACT TTG GTG CTT CTC ATT AAA 191
ALA SER GLN PHE LEU SER ALA LEU THR LEU VAL LEU LEU ILE LYS
5 10 15

GAG AGT GGA GCC TGG TCT TAC AAC ACC TCC ACG GAA GCT ATG ACT 236

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GLU SER GLY ALA TRP SER TYR ASN THR SER THR GLU ALA MET THR
        20                  25                  30
TAT GAT GAG GCC AGT GCT TAT TGT CAG CAA AGG TAC ACA CAC CTG 281
TYR ASP GLU ALA SER ALA TYR CYS GLN GLN ARG TYR THR HIS LEU
        35                  40                  45
GTT GCA ATT CAA AAC AAA GAA GAG ATT GAG TAC CTA AAC TCC ATA 326
VAL ALA ILE GLN ASN LYS GLU GLU ILE GLU TYR LEU ASN SER ILE
        50                  55                  60
TTG AGC TAT TCA CCA AGT TAT TAC TGG ATT GGA ATC AGA AAA GTC 371
LEU SER TYR SER PRO SER TYR TYR TRP ILE GLY ILE ARG LYS VAL
        65                  70                  75
AAC AAT GTG TGG GTC TGG GTA GGA ACC CAG AAA CCT CTG ACA GAA 416
ASN ASN VAL TRP VAL TRP VAL GLY THR GLN LYS PRO LEU THR GLU
        80                  85                  90
GAA GCC AAG AAC TGG GCT CCA GGT GAA CCC AAC AAT AGG CAA AAA 461
GLU ALA LYS ASN TRP ALA PRO GLY GLU PRO ASN ASN ARG GLN LYS
        95                  100                 105
GAT GAG GAC TGC GTG GAG ATC TAC ATC AAG AGA GAA AAA GAT GTG 506
ASP GLU ASP CYS VAL GLU ILE TYR ILE LYS ARG GLU LYS ASP VAL
        110                 115                 120
GGC ATG TGG AAT GAT GAG AGG TGC AGC AAG AAG AAG CTT GCC CTA 551
GLY MET TRP ASN ASP GLU ARG CYS SER LYS LYS LYS LEU ALA LEU
        125                 130                 135
TGC TAC ACA GCT GCC TGT ACC AAT ACA TCC TGC AGT GGC CAC GGT 596
CYS TYR THR ALA ALA CYS THR ASN THR SER CYS SER GLY HIS GLY
        140                 145                 150
GAA TGT GTA GAG ACC ATC AAT AAT TAC ACT TGC AAG TGT GAC CCT 641
GLU CYS VAL GLU THR ILE ASN ASN TYR THR CYS LYS CYS ASP PRO
        155                 160                 165
GGC TTC AGT GGA CTC AAG TGT GAG CAA ATT GTG AAC TGT ACA GCC 686
GLY PHE SER GLY LEU LYS CYS GLU GLN ILE VAL ASN CYS THR ALA
        170                 175                 180
CTG GAA TCC CCT GAG CAT GGA AGC CTG GTT TGC AGT CAC CCA CTG 731
LEU GLU SER PRO GLU HIS GLY SER LEU VAL CYS SER HIS PRO LEU
        185                 190                 195
GGA AAC TTC AGC TAC AAT TCT TCC TGC TCT ATC AGC TGT GAT AGG 776
GLY ASN PHE SER TYR ASN SER SER CYS SER ILE SER CYS ASP ARG
        200                 205                 210
GGT TAC CTG CCA AGC AGC ATG GAG ACC ATG CAG TGT ATG TCC TCT 821
GLY TYR LEU PRO SER SER MET GLU THR MET GLN CYS MET SER SER
        215                 220                 225
GGA GAA TGG AGT GCT CCT ATT CCA GCC TGC AAT GTG GTT GAG TGT 866
GLY GLU TRP SER ALA PRO ILE PRO ALA CYS ASN VAL VAL GLU CYS
        230                 235                 240
GAT GCT GTG ACA AAT CCA GCC AAT GGG TTC GTG GAA TGT TTC CAA 911
ASP ALA VAL THR ASN PRO ALA ASN GLY PHE VAL GLU CYS PHE GLN
        245                 250                 255
AAC CCT GGA AGC TTC CCA TGG AAC ACA ACC TGT ACA TTT GAC TGT 956
ASN PRO GLY SER PHE PRO TRP ASN THR THR CYS THR PHE ASP CYS
        260                 265                 270
GAA GAA GGA TTT GAA CTA ATG GGA GCC CAG AGC CTT CAG TGT ACC 1001
GLU GLU GLY PHE GLU LEU MET GLY ALA GLN SER LEU GLN CYS THR
        275                 280                 285
TCA TCT GGG AAT TGG GAC AAC GAG AAG CCA ACG TGT AAA GCT GTG 1046
SER SER GLY ASN TRP ASP ASN GLU LYS PRO THR CYS LYS ALA VAL
        290                 295                 300
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ACA TGC AGG GCC GTC CGC CAG CCT CAG AAT GGC TCT GTG AGG TGC 1091
THR CYS ARG ALA VAL ARG GLN PRO GLN ASN GLY SER VAL ARG CYS
305 310 315

AGC CAT TCC CCT GCT GGA GAG TTC ACC TTC AAA TCA TCC TGC AAC 1136
SER HIS SER PRO ALA GLY GLU PHE THR PHE LYS SER SER CYS ASN
320 325 330

TTC ACC TGT GAG GAA GGC TTC ATG TTG CAG GGA CCA GCC CAG GTT 1181
PHE THR CYS GLU GLU GLY PHE MET LEU GLN GLY PRO ALA GLN VAL
335 360 370

GAA TGC ACC ACT CAA GGG CAG TGG ACA CAG CAA ATC CCA GTT TGT 1226
GLU CYS THR THR GLN GLY GLN TRP THR GLN GLN ILE PRO VAL CYS
375 380 385

GAA GCT TTC CAG TGC ACA GCC TTG TCC AAC CCC GAG CGA GGC TAC 1271
GLU ALA PHE GLN CYS THR ALA LEU SER ASN PRO GLU ARG GLY TYR
390 395 400

ATG AAT TGT CTT CCT AGT GCT TCT GGC AGT TTC CGT TAT GGG TCC 1316
MET ASN CYS LEU PRO SER ALA SER GLY SER PHE ARG TYR GLY SER
405 410 415

AGC TGT GAG TTC TCC TGT GAG CAG GGT TTT GTG TTG AAG GGA TCC 1361
SER CYS GLU PHE SER CYS GLU GLN GLY PHE VAL LEU LYS GLY SER
420 425 430

AAA AGG CTC CAA TGT GGC CCC ACA GGG GAG TGG GAC AAC GAG AAG 1406
LYS ARG LEU GLN CYS GLY PRO THR GLY GLU TRP ASP ASN GLU LYS
435 440 445

CCC ACA TGT GAA GCT GTG AGA TGC GAT GCT GTC CAC CAG CCC CCG 1451
PRO THR CYS GLU ALA VAL ARG CYS ASP ALA VAL HIS GLN PRO PRO
450 455 460

AAG GGT TTG GTG AGG TGT GCT CAT TCC CCT ATT GGA GAA TTC ACC 1496
LYS GLY LEU VAL ARG CYS ALA HIS SER PRO ILE GLY GLU PHE THR
465 470 475

TAC AAG TCC TCT TGT GCC TTC AGC TGT GAG GAG GGA TTT GAA TTA 1541
TYR LYS SER SER CYS ALA PHE SER CYS GLU GLU GLY PHE GLU LEU
480 485 490

TAT GGA TCA ACT CAA CTT GAG TGC ACA TCT CAG GGA CAA TGG ACA 1586
TYR GLY SER THR GLN LEU GLU CYS THR SER GLN GLY GLN TRP THR
495 500 505

GAA GAG GTT CCT TCC TGC CAA GTG GTA AAA TGT TCA AGC CTG GCA 1631
GLU GLU VAL PRO SER CYS GLN VAL VAL LYS CYS SER SER LEU ALA
510 515 520

GTT CCG GGA AAG ATC AAC ATG AGC TGC AGT GGG GAG CCC GTG TTT 1676
VAL PRO GLY LYS ILE ASN MET SER CYS SER GLY GLU PRO VAL PHE
525 530 535

GGC ACT GTG TGC AAG TTC GCC TGT CCT GAA GGA TGG ACG CTC AAT 1721
GLY THR VAL CYS LYS PHE ALA CYS PRO GLU GLY TRP THR LEU ASN
540 545 550

GGC TCT GCA GCT CGG ACA TGT GGA GCC ACA GGA CAC TGG TCT GGC 1766
GLY SER ALA ALA ARG THR CYS GLY ALA THR GLY HIS TRP SER GLY
555 560 565

CTG CTA CCT ACC TGT GAA GCT CCC ACT GAG TCC AAC ATT CCC TTG 1811
LEU LEU PRO THR CYS GLU ALA PRO THR GLU SER ASN ILE PRO LEU
570 575 580

GTA GCT GGA CTT TCT GCT GCT GGA CTC TCC CTC CTG ACA TTA GCA 1856
VAL ALA GLY LEU SER ALA ALA GLY LEU SER LEU LEU THR LEU ALA
585 590 595

CCA TTT CTC CTC TGG CTT CGG AAA TGC TTA CGG AAA GCA AAG AAA 1901
PRO PHE LEU LEU TRP LEU ARG LYS CYS LEU ARG LYS ALA LYS LYS

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        600                           605                           610
TTT GTT CCT GCC AGC AGC TGC CAA AGC CTT GAA TCA GAC GGA AGC 1946
PHE VAL PRO ALA SER SER CYS GLN SER LEU GLU SER ASP GLY SER
        615                           620                           625
TAC CAA AAG CCT TCT TAC ATC CTT TAA GTTCAAA AGAATCAGAA      1990
TYR GLN LYS PRO SER TYR ILE LEU ***
        630                           635

ACAGGTGCAT CTGGGGAACT AGAGGGATAC ACTGAAGTTA ACAGAGACAG      2040

ATAACTCTCC TCGGGTCTCT GGCCCTTCTT GCCTACTATG CCAGATGCCT      2090

TTATGGCTGA AACCGCAACA CCCATCACCA CTTCAATAGA TCAAAGTCCA      2140

GCAGGCAAGG ACGGCCTTCA ACTGAAAAGA CTCAGTGTTC CCTTTCCTAC      2190

TCTCAGGATC AAGAAAGTGT TGGCTAATGA AGGGAAAGGA TATTTTCTTC      2240

CAAGCAAAGG TGAAGAGACC AAGACTCTGA AATCTCAGAA TTCCTTTTCT      2290

AACTCTCCCT TGCTCGCTGT AAAATCTTGG CACAGAAACA CAATATTTTG      2340

TGGCTTTCTT TCTTTTGCCC TTCACAGTGT TTCGACAGCT GATTACACAG      2390

TTGCTGTCAT AAGAATGAAT AATAATTATC CAGAGTTTAG AGGAAAAAAA      2440

TGACTAAAAA TATTATAACT TAAAAAAATG ACAGATGTTG AATGCCCACA      2490

GGCAAATGCA TGGAGGGTTG TTAATGGTGC AAATCCTACT GAATGCTCTG      2540

TGCGAGGGTT ACTATGCACA ATTTAATCAC TTTCATCCCT ATGGGATTCA      2590

GTGCTTCTTA AAGAGTTCTT AAGGATTGTG ATATTTTTAC TTGCATTGAA      2640

TATATTATAA TCTTCCATAC TTCTTCATTC AATACAAGTG TGGTAGGGAC      2690

TTAAAAAACT TGTAAATGCT GTCAACTATG ATATGGTAAA AGTTACTTAT      2740

TCTAGATTAC CCCCTCATTG TTTATTAACA AATTATGTTA CATCTGTTTT      2790

AAATTTATTT CAAAAAGGGA AACTATTGTC CCCTAGCAAG GCATGATGTT      2840

AACCAGAATA AAGTTCTGAG TGTTTTTACT ACAGTTGTTT TTTGAAAACA      2890

TGGTAGAATT GGAGAGTAAA AACTGAATGG AAGGTTTGTA TATTGTCAGA      2940

TATTTTTTCA GAAATATGTG GTTTCCACGA TGAAAAACTT CCATGAGGCC      2990

AAACGTTTTG AACTAATAAA AGCATAAATG CAAACACACA AAGGTATAAT      3040

TTTATGAATG TCTTTGTTGG AAAAGAATAC AGAAAGATGG ATGTGCTTTG      3090

CATTCCTACA AAGATGTTTG TCAGATGTGA TATGTAAACA TAATTCTTGT      3140

ATATTATGGA AGATTTTAAA TTCACAATAG AAACTCACCA TGTAAAAGAG      3190
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TCATCTGGTA GATTTTTAAC GAATGAAGAT GTCTAATAGT TATTCCCTAT 3240

TTGTTTTCTT CTGTATGTTA GGGTGCTCTG GAAGAGAGGA ATGCCTGTGT 3290

GAGCAAGCAT TTATGTTTAT TTATAAGCAG ATTTAACAAT TCCAAAGGAA 3340

TCTCCAGTTT TCAGTTGATC ACTGGCAATG AAAAATTCTC AGTCAGTAAT 3390

TGCCAAAGCT GCTCTAGCCT TGAGGAGTGT GAGAATCAAA ACTCTCCTAC 3440

ACTTCCATTA ACTTAGCATG TGTTGAAAAA AAAAGTTTCA GAGAAGTTCT 3490

GGCTGAACAC TGGCAACGAC AAAGCCAACA GTCAAAACAG AGATGTGATA 3540

AGGATCAGAA CAGCAGAGGT TCTTTTAAAG GGGCAGAAAA ACTCTGGGAA 3590

ATAAGAGAGA ACAACTACTG TGATCAGGCT ATGTATGGAA TACAGTGTTA 3640

TTTTCTTTGA AATTGTTTAA GTGTTGTAAA TATTTATGTA AACTGCATTA 3690

GAAATTAGCT GTGTGAAATA CCAGTGTGGT TTGTGTTTGA GTTTTATTGA 3740

GAATTTTAAA TTATAACTTA AAATATTTTA TAATTTTTAA AGTATATATT 3790

TATTTAAGCT TATGTCAGAC CTATTTGACA TAACACTATA AAGGTTGACA 3840

ATAAATGTGC TTATGTTT 3858

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2813

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

CGGGCCTCAC TGGCTTCAGG AGCTGAATAC CCTCCCAGGC ACACACAGGT 50

GGGACACAAA TAAGGGTTTT GGAACCACTA TTTTCTCATC ACGACAGCAA 100

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CTTAAA ATG CCT GGG AAG ATG GTC GTG ATC CTT GGA GCC     139
       MET PRO GLY LYS MET VAL VAL ILE LEU GLY ALA
                        5                  10
TCA AAT ATA CTT TGG ATA ATG TTT GCA GCT TCT CAA GCT TTT AAA 184
SER ASN ILE LEU TRP ILE MET PHE ALA ALA SER GLN ALA PHE LYS
        15                  20                  25
ATC GAG ACC ACC CCA GAA TCT AGA TAT CTT GCT CAG ATT GGT GAC 229
ILE GLU THR THR PRO GLU SER ARG TYR LEU ALA GLN ILE GLY ASP
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                30                      35                      40
TCC GTC TCA TTG ACT TGC AGC ACC ACA GGC TGT GAG TCC CCA TTT 274
SER VAL SER LEU THR CYS SER THR THR GLY CYS GLU SER PRO PHE
                45                      50                      55
TTC TCT TGG AGA ACC CAG ATA GAT AGT CCA CTG AAT GGG AAG GTG 319
PHE SER TRP ARG THR GLN ILE ASP SER PRO LEU ASN GLY LYS VAL
                60                      65                      70
ACG AAT GAG GGG ACC ACA TCT ACG CTG ACA ATG AAT CCT GTT AGT 364
THR ASN GLU GLY THR THR SER THR LEU THR MET ASN PRO VAL SER
                75                      80                      85
TTT GGG AAC GAA CAC TCT TAC CTG TGC ACA GCA ACT TGT GAA TCT 409
PHE GLY ASN GLU HIS SER TYR LEU CYS THR ALA THR CYS GLU SER
                90                      95                      100
AGG AAA TTG GAA AAA GGA ATC CAG GTG GAG ATC TAC TCT TTT CCT 454
ARG LYS LEU GLU LYS GLY ILE GLN VAL GLU ILE TYR SER PHE PRO
                105                     110                     115
AAG GAT CCA GAG ATT CAT TTG AGT GGC CCT CTG GAG GCT GGG AAG 499
LYS ASP PRO GLU ILE HIS LEU SER GLY PRO LEU GLU ALA GLY LYS
                120                     125                     130
CCG ATC ACA GTC AAG TGT TCA GTT GCT GAT GTA TAC CCA TTT GAC 544
PRO ILE THR VAL LYS CYS SER VAL ALA ASP VAL TYR PRO PHE ASP
                135                     140                     145
AGG CTG GAG ATA GAC TTA CTG AAA GGA GAT CAT CTC ATG AAG AGT 589
ARG LEU GLU ILE ASP LEU LEU LYS GLY ASP HIS LEU MET LYS SER
                150                     155                     160
CAG GAA TTT CTG GAG GAT GCA GAC AGG AAG TCC CTG GAA ACC AAG 634
GLN GLU PHE LEU GLU ASP ALA ASP ARG LYS SER LEU GLU THR LYS
                165                     170                     175
AGT TTG GAA GTA ACC TTT ACT CCT GTC ATT GAG GAT ATT GGA AAA 679
SER LEU GLU VAL THR PHE THR PRO VAL ILE GLU ASP ILE GLY LYS
                180                     185                     190
GTT CTT GTT TGC CGA GCT AAA TTA CAC ATT GAT GAA ATG GAT TCT 724
VAL LEU VAL CYS ARG ALA LYS LEU HIS ILE ASP GLU MET ASP SER
                195                     200                     205
GTG CCC ACA GTA AGG CAG GCT GTA AAA GAA TTG CAA GTC TAC ATA 769
VAL PRO THR VAL ARG GLN ALA VAL LYS GLU LEU GLN VAL TYR ILE
                210                     215                     220
TCA CCC AAG AAT ACA GTT ATT TCT GTG AAT CCA TCC ACA AAG CTG 814
SER PRO LYS ASN THR VAL ILE SER VAL ASN PRO SER THR LYS LEU
                225                     230                     235
CAA GAA GGT GGC TCT GTG ACC ATG ACC TGT TCC AGC GAG GGT CTA 859
GLN GLU GLY GLY SER VAL THR MET THR CYS SER SER GLU GLY LEU
                240                     245                     250
CCA.GCT CCA GAG ATT TTC TGG AGT AAG AAA TTA GAT AAT GGG AAT 904
PRO ALA PRO GLU ILE PHE TRP SER LYS LYS LEU ASP ASN GLY ASN
                255                     260                     265
CTA CAG CAC CTT TCT GGA AAT GCA ACT CTC ACC TTA ATT GCT ATG 949
LEU GLN HIS LEU SER GLY ASN ALA THR LEU THR LEU ILE ALA MET
                270                     275                     280
AGG ATG GAA GAT TCT GGA ATT TAT GTG TGT GAA GGA GTT AAT TTG 994
ARG MET GLU ASP SER GLY ILE TYR VAL CYS GLU GLY VAL ASN LEU
                285                     290                     295
ATT GGG AAA AAC AGA AAA GAG GTG GAA TTA ATT GTT CAA GCA TTC 1039
ILE GLY LYS ASN ARG LYS GLU VAL GLU LEU ILE VAL GLN ALA PHE
                300                     305                     310
CCT AGA GAT CCA GAA ATC GAG ATG AGT GGT GGC CTC GTG AAT GGG 1084
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PRO ARG ASP PRO GLU ILE GLU MET SER GLY GLY LEU VAL ASN GLY
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AGC TCT GTC ACT GTA AGC TGC AAG GTT CCT AGC GTG TAC CCC CTT 1129
SER SER VAL THR VAL SER CYS LYS VAL PRO SER VAL TYR PRO LEU
        330                 335                 340
GAC CGG CTG GAG ATT GAA TTA CTT AAG GGG GAG ACT ATT CTG GAG 1174
ASP ARG LEU GLU ILE GLU LEU LEU LYS GLY GLU THR ILE LEU GLU
        345                 350                 355
AAT ATA GAG TTT TTG GAG GAT ACG GAT ATG AAA TCT CTA GAG AAC 1219
ASN ILE GLU PHE LEU GLU ASP THR ASP MET LYS SER LEU GLU ASN
        360                 365                 370
AAA AGT TTG GAA ATG ACC TTC ATC CCT ACC ATT GAA GAT ACT GGA 1264
LYS SER LEU GLU MET THR PHE ILE PRO THR ILE GLU ASP THR GLY
        375                 380                 385
AAA GCT CTT GTT TGT CAG GCT AAG TTA CAT ATT GAT GAC ATG GAA 1309
LYS ALA LEU VAL CYS GLN ALA LYS LEU HIS ILE ASP ASP MET GLU
        390                 395                 400
TTC GAA CCC AAA CAA AGG CAG AGT ACG CAA ACA CTT TAT GTC AAT 1354
PHE GLU PRO LYS GLN ARG GLN SER THR GLN THR LEU TYR VAL ASN
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GTT GCC CCC AGA GAT ACA ACC GTC TTG GTC AGC CCT TCC TCC ATC 1399
VAL ALA PRO ARG ASP THR THR VAL LEU VAL SER PRO SER SER ILE
        420                 425                 430
CTG GAG GAA GGC AGT TCT GTG AAT ATG ACA TGC TTG AGC CAG GGC 1444
LEU GLU GLU GLY SER SER VAL ASN MET THR CYS LEU SER GLN GLY
        435                 440                 445
TTT CCT GCT CCG AAA ATC CTG TGG AGC AGG CAG CTC CCT AAC GGG 1489
PHE PRO ALA PRO LYS ILE LEU TRP SER ARG GLN LEU PRO ASN GLY
        450                 455                 460
GAG CTA CAG CCT CTT TCT GAG AAT GCA ACT CTC ACC TTA ATT TCT 1534
GLU LEU GLN PRO LEU SER GLU ASN ALA THR LEU THR LEU ILE SER
        465                 470                 475
ACA AAA ATG GAA GAT TCT GGG GTT TAT TTA TGT GAA GGA ATT AAC 1579
THR LYS MET GLU ASP SER GLY VAL TYR LEU CYS GLU GLY ILE ASN
        480                 485                 490
CAG GCT GGA AGA AGC AGA AAG GAA GTG GAA TTA ATT ATC CAA GTT 1624
GLN ALA GLY ARG SER ARG LYS GLU VAL GLU LEU ILE ILE GLN VAL
        495                 500                 505
ACT CCA AAA GAC ATA AAA CTT ACA GCT TTT CCT TCT GAG AGT GTC 1669
THR PRO LYS ASP ILE LYS LEU THR ALA PHE PRO SER GLU SER VAL
        510                 515                 520
AAA GAA GGA GAC ACT GTC ATC ATC TCT TGT ACA TGT GGA AAT GTT 1714
LYS GLU GLY ASP THR VAL ILE ILE SER CYS THR CYS GLY ASN VAL
        525                 530                 535
CCA GAA ACA TGG ATA ATC CTG AAG AAA AAA GCG GAG ACA GGA GAC 1759
PRO GLU THR TRP ILE ILE LEU LYS LYS LYS ALA GLU THR GLY ASP
        540                 545                 550
ACA GTA CTA AAA TCT ATA GAT GGC GCC TAT ACC ATC CGA AAG GCC 1804
THR VAL LEU LYS SER ILE ASP GLY ALA TYR THR ILE ARG LYS ALA
        555                 560                 565
CAG TTG AAG GAT GCG GGA GTA TAT GAA TGT GAA TCT AAA AAC AAA 1849
GLN LEU LYS ASP ALA GLY VAL TYR GLU CYS GLU SER LYS ASN LYS
        570                 575                 580
GTT GGC TCA CAA TTA AGA AGT TTA ACA CTT GAT GTT CAA GGA AGA 1894
VAL GLY SER GLN LEU ARG SER LEU THR LEU ASP VAL GLN GLY ARG
        585                 590                 595
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GAA AAC AAC AAA GAC TAT TTT TCT CCT GAG CTT CTC GTG CTC TAT 1939
GLU ASN ASN LYS ASP TYR PHE SER PRO GLU LEU LEU VAL LEU TYR
        600                 605                 610
TTT GCA TCC TCC TTA ATA ATA CCT GCC ATT GGA ATG ATA ATT TAC 1984
PHE ALA SER SER LEU ILE ILE PRO ALA ILE GLY MET ILE ILE TYR
        615                 620                 625
TTT GCA AGA AAA GCC AAC ATG AAG GGG TCA TAT AGT CTT GTA GAA 2029
PHE ALA ARG LYS ALA ASN MET LYS GLY SER TYR SER LEU VAL GLU
        630                 635                 640
GCA CAG AAA TCA AAA GTG TAG                                 2050
ALA GLN LYS SER LYS VAL ***
        645

CTAATGCTTG ATATGTTCAA CTGGAGACAC TATTTATCTG TGCAAATCCT      2100

TGATACTGCT CATCATTCCT TGAGAAAAAC AATGAGCTGA GAGGCAGACT      2150

TCCCTGAATG TATTGAACTT GGAAAGAAAT GCCCATCTAT GTCCCTTGCT      2200

GTGAGCAAGA AGTCAAAGTA AAACTTGCTG CCTGAAGAAC AGTAACTGCC      2250

ATCAAGATGA GAGAACTGGA GGAGTTCCTT GATCTGTATA TACAATAACA      2300

TAATTTGTAC ATATGTAAAA TAAAATTATG CCATAGCAAG ATTGCTTAAA      2350

ATAGCAACAC TCTATATTTA GATTGTTAAA ATAACTAGTG TTGCTTGGAC      2400

TATTATAATT TAATGCATGT TAGGAAAATT TCACATTAAT ATTTGCTGAC      2450

AGCTGACCTT TGTCATCTTT CTTCTATTTT ATTCCCTTTC ACAAAATTTT      2500

ATTCCTATAT AGTTTATTGA CAATAATTTC AGGTTTTGTA AAGATGCCGG      2550

GTTTTATATT TTTATAGACA AATAATAAGC AAAGGGAGCA CTGGGTTGAC      2600

TTTCAGGTAC TAAATACCTC AACCTATGGT ATAATGGTTG ACTGGGTTTC      2650

TCTGTATAGT ACTGGCATGG TACGGAGATG TTTCACGAAG TTTGTTCATC      2700

AGACTCCTGT GCAACTTTCC CAATGTGGCC TAAAAATGCA ACTTCTTTTT      2750

ATTTTCTTTT GTAAATGTTT AGGTTTTTTT GTATAGTAAA GTGATAATTT      2800

CTGGAATTAA AAA                                              2813
```

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

AGGGCCACTG CTCGTCCACA 20

What is claimed is:

1. A composition for treating allograft rejection comprising an oligonucleotide 8 to 50 nucleotides in length which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1 in combination with an immunosuppressive agent.

2. The composition of claim 1 wherein the oligonucleotide comprises SEQ ID NO: 22.

3. The composition of claim 1 wherein the immunosuppressive agent is a monoclonal antibody.

4. The composition of claim 2 wherein the monoclonal antibody is directed against LFA-1.

5. The composition of claim 1 wherein the immunosuppressive agent is brequinar, rapamycin or anti-lymphocyte serum.

6. The composition of claim 1 wherein the immunosuppressive agent is an antisense oligonucleotide.

7. A method of preventing allograft rejection in an allograft recipient comprising treating the allograft recipient with an oligonucleotide 8 to 50 nucleotides in length which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1, in combination with an immunosuppressive agent.

8. The method of claim 7 wherein the immunosuppressive agent is a monoclonal antibody.

9. The method of claim 8 wherein the monoclonal antibody is directed against LFA-1.

10. The method of claim 7 wherein the immunosuppressive agent is brequinar, rapamycin or anti-lymphocyte serum.

11. The method of claim 7 wherein the immunosuppressive agent is an antisense oligonucleotide.

12. The method of claim 7 wherein the allograft is a cardiac allograft.

13. The method of claim 7 wherein the allograft is a renal allograft.

14. A method of preventing allograft rejection in an allograft recipient comprising treating the allograft recipient with a composition of claim 1.

15. A method of preventing rejection of an allograft by an allograft recipient comprising treating the allograft with a composition of claim 1.

16. The method of claim 15 wherein the treatment is performed *ex vivo*.

17. A method of preventing rejection of an allograft comprising treating the allograft with an oligonucleotide 8 to 50 nucleotides in length which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1.

18. The method of claim 17 wherein the oligonucleotide comprises SEQ ID NO: 22.

19. A method of treating allograft rejection in an allograft recipient comprising treating the allograft recipient with an oligonucleotide 8 to 50 nucleotides in length which is targeted to a nucleic acid sequence encoding ICAM-1, ELAM-1 or VCAM-1 in combination with an immunosuppressive agent.

20. The method of claim 19 wherein the immunosuppressive agent is a monoclonal antibody.

21. The method of claim 20 wherein the monoclonal antibody is directed against LFA-1.

22. The method of claim 19 wherein the immunosuppressive agent is brequinar, rapamycin or anti-lymphocyte serum.

23. The method of claim 19 wherein the immunosuppressive agent is an antisense oligonucleotide.

24. The method of claim 19 wherein the allograft is a cardiac allograft.

25. The method of claim 19 wherein the allograft is a renal allograft.

26. A method of treating allograft rejection in an allograft recipient comprising treating the allograft recipient with a composition of claim 1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/15536

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/00
US CL :514/44
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, A, 94/05333 (BENNET ET AL) 17 MARCH 1994, see entire document.	1-26
Y	JOURNAL OF CELLULAR BIOCHEMISTRY, Vol. 17, issued 1993, Bennett et al, "INHIBITION OF ICAM-1, VCAM-1, AND E-SELECTIN EXPRESSION WITH ANTISENSE OLIGONUCLEOTIDES", page 354, abstract C 500, see entire document.	1-26
Y	JOURNAL OF IMMUNOLOGY, Vol. 152, issued 1994, Bennett et al, "Inhibition of Endothelial Cell Adhesion Molecule Expression with Antisense Oligonucleotides", pages 3530-3540, see entire document.	1-26

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion f the international search	Date of mailing of the international search report
01 FEBRUARY 1996	15 FEB 1996

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	D. CURTIS HOGUE, JR.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF IMMUNOLOGY, Vol. 153, issued 1994, Stepkowski et al, "Blocking of Heart Allograft Rejection by Intercellular Adhesion Molecule-1 Antisense Oligonucleotides Alone or in Combination with Other Immunosuppressive Modalities", pages 5336-5346, see entire document.	1-26
Y, P	JOURNAL OF INVESTIGATIVE DERMATOLOGY, Vol. 104, issued 1995, Hertl et al, "Inhibition of Interferon-gamma-Induced Intercellular Adhesion Molecule-1 Expression on Human Keratinocytes by Phosphorothioate Antisense Oligodeoxynucleotides Is the Consequence of Antisense-Specific and Antisense-Non-Specific Effects", pages 813-818, see entire document.	1-26
Y	JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 266, No. 27, issued 25 September 1991, Chiang et al, "Antisense Oligonucleotides Inhibit Intercellular Adhesion Molecule 1 Expression by Two Distinct Mechanisms", pages 18162-18171, see entire document.	1-26
Y	JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY, Vol. 4, No. 6, issued December 1993, Lu et al, "Prevention and Treatment of Renal Allograft Rejection: New Therapeutic Approaches and New Insights Into Established Therapies", pages 1239-1256, see entire document.	1-26
A	ANNALS OF SURGERY, Vol. 219, No. 1, issued 1994, Heemann et al, "Adhesion Molecules and Transplantation", pages 4-12, see entire document.	1-26
Y	TRANSPLANTATION PROCEEDINGS, Vol. 25, No. 4, issued August 1993, Groth et al, "New Immunosuppressive Drugs in Transplantation", pages 2681-2683, see entire document.	1,5,7,10,19,22
Y	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, Vol. 696, issued 1993, Cramer et al, "The Use of Brequinar Sodium of Transplantation", pages 216-226, see entire document.	1,5,7,10,19,22
Y	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, Vol. 696, issued 1993, S. N. Sehgal, "Immunosuppressive Profile of Rapamycin", pages 1-8, see entire document.	1,5,7,10,19,22